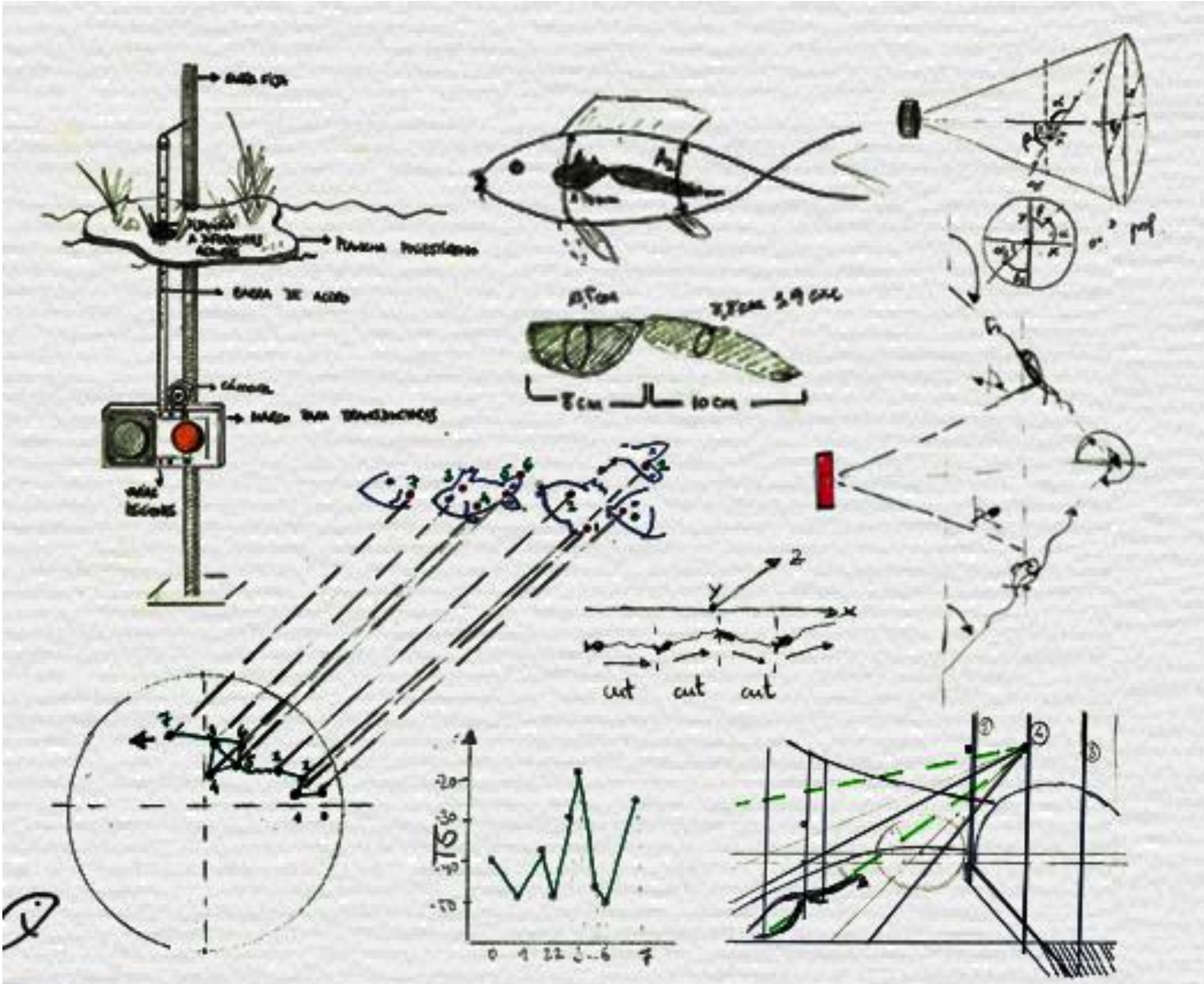

LA HIDROACÚSTICA HORIZONTAL APLICADA A LA DETECCIÓN DE PECES EN ECOSISTEMAS SOMEROS: ESTUDIO DE LA SEÑAL ACÚSTICA DE BARBOS Y CARPAS.

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Memoria para optar al título de Doctora en biología con Mención Europea por la Universidad de Sevilla

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Facultad de Biología

La hidroacústica horizontal aplicada a la detección de peces en ecosistemas someros: Estudio de la señal acústica de barbos y carpas.

Horizontal hydroacoustics applied to fish detection in shallow ecosystems: an acoustic study on barbel and carp

Memoria presentada por la licenciada Victoria Rodríguez Sánchez para optar al título de Doctora en biología con Mención Europea por la Universidad de Sevilla

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Sevilla, 2015

Organización de la memoria

La memoria consta de una introducción y un resumen de los resultados escritas en inglés. A continuación, se presentan los trabajos realizados para la memoria organizados por capítulos, cada uno de los cuales integra un artículo. Posteriormente, se presenta la discusión general y las conclusiones escritas en inglés. Al final del documento, se incluye la versión en castellano de la introducción, el resumen de los resultados, la discusión general y las conclusiones.

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Horizontal hydroacoustics applied to fish detection in shallow ecosystems: an acoustic study on barbel and carp

INTRODUCTION

1.- Hydroacoustics as a technique to study the ecology of fish

Studying and understanding the ecology of fish is not an easy task since they live in an environment that is unfamiliar to us and accessing the relevant information is difficult. Nevertheless, fish are an essential component of aquatic ecosystems and their study is fundamental for the knowledge thereof. The recognition of their importance is relatively recent since this group of vertebrates had always been forgotten by ecologists until the second half of the twentieth century. The classic explanation of the functioning of the ecosystems did not consider them regulating agents. The aquatic ecosystem was considered a functional machine where the primary producers directed their energy towards higher levels of predators (bottom-up control). Within this concept, fish did not seem to play an important role in regulating the system and, therefore, their study was very limited and generally descriptive (Granado-Lorencio, 2000; Encina *et al.*, 2006).

It was not until Brooks and Dodson's work (1965) that fish started gaining major importance as part of the ecosystems. Thanks to this work, a new theory appeared in which fish were considered to be at the centre of the functioning of the ecosystem (top-down control). Subsequently, Northcote (1988) summarised the role of fish in the ecosystem integrating both the classic bottom-up concept and the new top-down theory. According to this approach, predation controls the community structure while competition and resource availability limit the maximum production of each trophic level (Mills and Forney, 1988). This concept of trophic cascade combines the principles of limnology and fishery ecology and it states that the low levels of the trophic chain might be controlled by regulating the fish stocks in the aquatic environment (Carpenter *et al.*, 1985). From the publication of these works on, the study of fish communities started to gain importance. In this kind of study, several processes and aspects of fish biology must be analysed in order to obtain a holistic view of the system. It is necessary to know the fish's associations, their composition and regulation over time; their abundance and the role they play within the energy flow; their distribution in aquatic habitats and their relationships with other components of the system (prey and predators). Furthermore, it is also important to consider other issues related to the conservation and management of aquatic ecosystems.

Several methods exist to study fish populations, which can be broadly classified into two different groups: capture-dependent methods and capture-independent methods (Lucas and Baras, 2000). Capture-dependent methods include gill nets, trawl nets, purse seines, electric fishing, etc. These techniques offer a low sampling coverage and they do not provide information about the absolute values of the density and biomass of fish communities. Moreover, the information obtained by means of these methods may not represent the ecosystem properly due to the lack of homogeneity in fish distribution and to the influence of ethological aspects and those related to the selectivity of the fishing gears (Emmrich *et al.*, 2012). One of the most relevant capture-independent methods is hydroacoustics, which allows measuring fish distribution and abundance in a non-selective way. It is also quite useful when studying the behaviour and migration of species in both freshwater and marine systems (Steig and Iverson, 1998; Guillard 1998; Lucas and Baras 2000; Simmonds and MacLennan, 2005). Unlike imaging study techniques, hydroacoustics is efficient even when used in ecosystems with poor visibility. This is why many studies have highlighted its suitability to study aquatic ecosystems. Choosing not to use this technique can hardly be justified in terms of costs and benefits (Godlewska, 2004, Monteoliva and Schneider, 2005; Kubecka *et al.*, 2009).

One of the most important benefits of hydroacoustics is that it allows studying large water surfaces in a short time and analysing the information provided by different components of the ecosystem (Brandt, 1996; Wanzeböck *et al.*, 2003; Godlewska, 2004; Winfield *et al.*, 2007). Furthermore, it is a non-lethal technique that does not directly affect the organisms of the studied system. The most notable disadvantage is that species cannot be identified using hydroacoustics alone. Instead, it must be complemented with other techniques in order to obtain such information. Nevertheless, many studies recommend combining several sampling techniques in order to obtain the complete picture of the aquatic ecosystem. Jointly using several methods is a way to minimise the selectivity effect associated with traditional techniques and to complement the information obtained by each individual technique, which in the end offers improved results (Prchalova *et al.*, 2009; Winfield *et al.*, 2009; Harrison *et al.*, 2010; Emmrich *et al.*, 2012; Kubecka *et al.*, 2012).

Hydroacoustics is a technique in which sound is used to study aquatic ecosystems. Researchers have been fascinated by sound and how it moves through water for quite some time. In 1490, Leonardo Da Vinci stated: "If you cause your ship to stop and place the head of a long tube in the water and place the outer extremity to your ear, you will hear ships at a great distance from you". In 1826, Daniel Colladon, a Swiss physicist, and Charles Sturm, a French mathematician, accurately measured the speed of sound in water (Fig. 1). With the aid of a long tube to hear under water (as suggested by Da Vinci), they managed to register how long it took the sound of a bell placed under water to traverse all of Lake Geneva. The result was 1,435 m (1,569 yd) per second in water at 1.8 ºC (35 ºF), only 3 metres per second less than the speed accepted nowadays. What these investigators managed to prove is that water, either fresh or salt, constitutes an excellent means for sound. Sound transmission in water occurs almost five times faster than in the air (National Academy of Science, 2003, Washington D.C.) (http://www.nationalacademies.org).

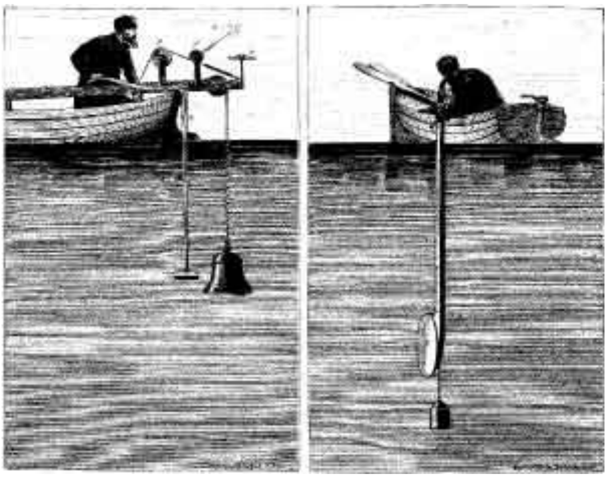

Figure 1. Charles Sturm (left) and Daniel Colladon (right) measuring the speed of sound in water for the first time (illustration property of the Acoustical Society of America).

It was not until the beginning of the twentieth century that hydroacoustics started having a practical application as a result of advances in technology. The discovery of piezoelectricity by Jacques and Pierre Curie in 1880 was fundamental for the development of the piezoelectric transmitter in 1917, which gave way to the first hydroacoustic experiences. The term "echosounding" was used for the first time in the 1920s. It referred to the technique used to measure the depth of water columns. A few years later, in 1929, Kimura performed the first successful experience in fish detection (Simmonds and Maclennan, 2005). In the 1970s and 1980s, with studies by Love (1977) and Foote (1980); Foote *et al.* (1987), the sound backscattered from fish was determined and equations relating the fish's backscattered energy to their length were established. These new associations were incorporated into the routines of hydroacoustic samplings, which gave promising results in fish biomass and density estimates. From then until now, intense theoretical and experimental research has been conducted, which has provided improved knowledge and has enabled a more appropriate application of these methods.

The European Committee for Standardization (CEN) has recently started to draw up a standard for the use of hydroacoustics (CEN, 2009). In order to help develop these regulations, certain matters regarding the functioning of hydroacoustics need to be sorted. This standardisation is aimed at providing tools to enable the comparison between data obtained in different water bodies using different systems.

Moreover, in order for the European Water Framework Directive (WFD) (2000/60/EC) to be implemented, the European states have to perform an assessment of all their water surfaces and ensure their good ecological conditions for the year 2015. This European directive requires that the results of the works performed on any aquatic ecosystem within the European Union be directly comparable. In general, data differ in terms of sampling techniques or applied systems. These methodological differences highly complicate the interpretation of data and their direct comparison (intercalibration). In the intercalibration process demanded by the WFD, these differences need to be analysed in order to define and unify the international criteria to be used when combining hydroacoustics with other methods or systems.

In this regard, some previous comparisons are already available (Wanzeböck *et al.*, 2003, Guillard *et al.*, 2004; Guillard and Vergès, 2007; Rakowitz *et al.*, 2008; Godlewska *et al.*, 2009). However, there are numerous topics that have yet to be studied. Therefore, a higher number of experiences are still required in order to achieve the standardisation of this methodology. Works such as those described here are intended to solve some of the problems associated with the use and application of horizontal hydroacoustics. They compare systems and frequencies in order to ease the comparison of hydroacoustic data obtained using different devices, which eases the intercalibration of systems.

2.- How does hydroacoustics work?

Hydroacoustics is a technique in which sound and its properties are applied to remotely detect and determine the position of objects submerged in aquatic ecosystems. In order to obtain this information, an "echosounder" is employed. This device works as a transmitter and a receiver of sound signals. It emits sound waves that travel through water colliding with every organism and particle that they encounter along the way. Each one of the obstacles that the sound waves collide with reflects an echo back. All reflected echoes are collected by the receiver and the data acquisition software translates them into an image (echogram) that represents the aquatic ecosystem (Fig. 2). Fish, as acoustic targets, return echoes as well. Their reflectivity is summarised in a parameter known as backscattering cross-section (σ_{bs}), which is essentially the acoustic size of the fish. This parameter is usually expressed in logarithmic form and it is known as target strength or TS (expressed in dB relative to 1 m^2) (Sunardi *et al.*, 2009).

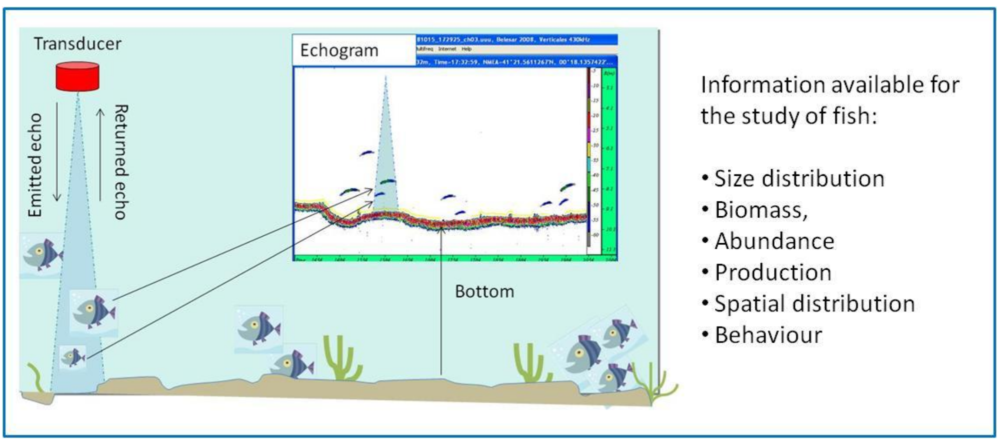

Figure 2. Explanation of the functioning of an echosounder and results of the sampling.

Therefore, the key to properly interpret the data obtained from hydroacoustic samplings is the target strength value returned by fish. This value allows creating conversion equations that translate the sound returned by fish (TS) into biological parameters that can be measured, such as length or weight. The use of these equations in the analysis of the information obtained from hydroacoustic samplings allows calculating the size, number and position of fish in an ecosystem (Lucas and Baras, 2000).

In general, the target strength value depends on several fish morphological parameters such as length, anatomical features, type and size of swim bladder, species, etc. It also depends on parameters that are not related to the fish's natural features such as the fish's position in the acoustic beam, the fish's swimming orientation, the ensonification system that is used (single, dual or split-beam), the applied frequency, etc. (Horne and Clay, 1998; Sawada *et al*., 2002; Hazen and Horne, 2003; Simmonds and MacLennan, 2005; Pedersen *et al.*, 2009; Jech 2011). All these features must be taken into consideration when creating the conversion equations. Therefore, these equations will be specific to the abovementioned parameters in the majority of cases.

Despite the fact that some conversion equations already exist for several species of freshwater fish, both for lateral positions and for other aspects (Borisenko *et al.*, 1989; Burwen and Fleischman, 1998; Lilja *et al.*, 2000; Frouzova and Kubecka, 2004; Knudsen *et al.*, 2004; Frouzova *et al.*, 2005; Boswell and Wilson, 2008), these are not sufficient to meet the needs of hydroacoustics due to the wide range of species, frequencies and types of echosounders used (Lucas and Baras, 2000).

3.- Horizontal hydroacoustics

Hydroacoustics has traditionally been applied in marine systems with the acoustic beam aimed vertically (perpendicular to the water surface) (Simmonds and MacLennan, 2005). In these environments, the technique has been thoroughly improved and developed. It has proven to be a useful sampling technique for studies of fish ecology (Lucas and Baras, 2000). Little by little, it has also started to be employed in freshwater ecosystems (Brandt, 1996), where problems other than those found in marine environments have been identified. Notable among these is that vertical sampling encounters some limitations when used in shallow systems. In their studies, Kubecka and Wittingerova (1998) and Knudsen and Saegrov (2002) warned of a possible underestimation of the density of ecosystems resulting from the inability to quantify the number of fish found in superficial and shallow habitats. Certainly, the acoustic beam is so small in the superficial layers that vertical samplings are not enough to cover these areas. This technique encounters problems interpreting data from these areas due to the so-called hydroacoustic blind zone. To resolve these limitations, both studies suggest complementing the information obtained from vertical samplings with data acquired using horizontal hydroacoustics (with the beam parallel to the water surface). Horizontal applications are usually employed to study shallow systems such as rivers or superficial layers in large deep water systems since a large number of fish use these habitats as a refuge, a feeding area, etc. (Encina *et al.*, 2006; Kubecka *et al.*, 2012). However, horizontal hydroacoustics is less developed than vertical hydroacoustics and it needs to be studied in more detail in order to resolve the issues that emerge with increased use.

Horizontal hydroacoustics requires specific equations different from those employed in vertical applications. These new equations must include information about the fish's swimming orientation (Hazen and Horne, 2003; Simmonds and MacLennan, 2005; Pedersen *et al.*, 2009; Jech, 2011, Rodríguez-Sánchez *et al.*, 2015a, b). The scarcity of equations in vertical hydroacoustics is even greater in horizontal hydroacoustics since its use is very recent and there are only a few conversions specific to particular species and hydroacoustic systems (Burwen and Fleischman, 1998; Lilja *et al.*, 2000; Frouzova and Kubecka, 2004; Frouzova *et al.*, 2005; Boswell and Wilson, 2008).

In order to experimentally develop TS equations, it is best to use free-swimming fish since the results will be more similar to the values found in nature (Simmonds and

MacLennan, 2005). However, the use of free-swimming fish presents particular problems associated with the time required to obtain quality data and with determining the fish’s swimming angle, for which there is currently no established method of calculation. Some authors use the information provided by visual media such as pictures (Huse and Ona, 1996), while others employ the XY positions of the echoes of the swimming tracks and the fish's movement speed (Pederson *et al.*, 2009). Balk and Lindem (2011) developed different methods to calculate fish orientation depending on the researcher's needs. However, there are no agreed methods to calculate fish's swimming angles with horizontal orientations. Furthermore, the large amount of time required to form conversion equations can be a limiting factor. Collecting acoustic data from free-swimming fish is very complicated and the linear tracks must be manually selected (Simmonds and MacLennan, 2005), which renders the data acquisition process highly time-consuming.

Article I (**Horizontal target strength of *Luciobarbus sp.* in *ex-situ* experiments: Testing differences by aspect angle, pulse length and beam position**) presents horizontal conversion equations for barbel developed with a split-beam system operating at 200 kHz. This genus is one of the most important in Iberian Peninsula fish communities. Furthermore, it is widely distributed in Africa, Asia and Europe, where it is the dominant component in the cyprinid fauna. Despite its wide distribution, there is no information about its acoustical properties. Therefore, there is an urgent need to perform studies thereof in order to improve the results of the ecological studies performed in these systems.

Moreover, this article presents the analysis results of the problems identified when forming the horizontal conversion equations in order to find solutions and ease their development. A new method has been developed to determine the fish’s swimming angle. This method calculates fish orientation integrating all of the moving fish's backscattered energy in a regression line. In order to ensure the goodness of fit, the results have been compared with videos simultaneously recorded during the ensonification process. The behaviour of the backscattered TS has also been studied in different positions in the acoustic beam. The aim was to extend the usable area for data acquisition, thereby reducing the time required to create conversion equations.

Besides the conversion equations, there is another parameter selected at the beginning of the sampling that determines the efficiency of the technique: the pulse length. This term refers to the duration of a sonar transmission pulse. This parameter has an influence on the discrimination capacity of the system when fish are close to each other. If the pulse length is too long, the transmitted burst of sound could encompass two or more fish at the same time and the system would interpret that as a single fish. In that case, selecting a shorter pulse length would allow including each fish in a different burst of sound so that the system could interpret each of them as individual fish (Figure 3). Therefore, when fish density is high, the selected pulse lengths should be shorter since they allow for a better identification of individual fish. Previous studies have shown that no differences seem to exist in the fish's backscattered TS when using shorter pulse lengths (0.1-0.4 ms) (Kubecka, 1995; Boswell and Wilson, 2008; Godlewska *et al.*, 2011). However, these studies used systems and frequencies other than those applied in this work. Therefore, we have considered the possibility of comparing this information with that obtained using our 200 kHz split-beam system. To perform this comparison, the TS values for *Luciobarbus sp.* were studied using two different pulse lengths.

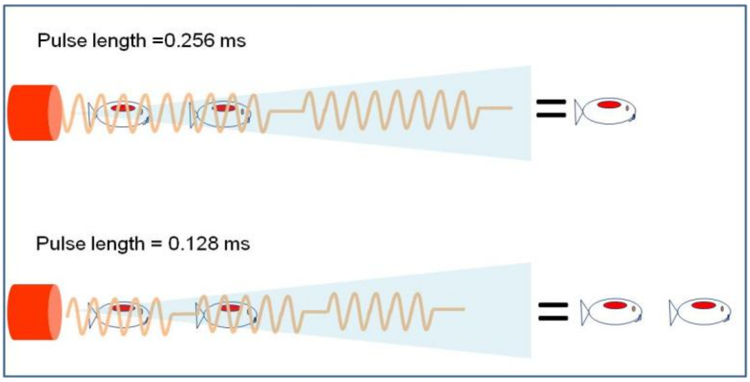

Figure 3. Graphic explanation of the effect of pulse length on the detection of adjacent fish.

The use and application of horizontal hydroacoustics give way to new problems associated with data acquisition. Due to the wide variety of echosounders (single, dual or split-beam), frequencies and acquisition parameters, choosing the correct system and the appropriate parameters will be based on the features of the aquatic ecosystems, the organisms to be studied and the existing information about the functioning of the hydroacoustic tools.

With regards to the selection of the system, horizontal hydroacoustics requires information about the position of fish. Therefore, the selected systems must use two or more beams. Dual and split-beam systems are very useful when performing this type of sampling, although they work in different ways. Dual-beam systems use transducers that emit sound in two concentric beams that work alternately, allowing the correction of acoustic signals (echoes) depending on distance and position. Split-beam systems have a transducer divided into four quadrants and the target direction is determined by comparing the signals received by each quadrant. When comparing the sound information received from each quadrant, it is possible to identify and position the individual targets in a three-dimensional space within the sound beam. Although both systems are very useful to study shallow ecosystems, there are several issues that should be previously taken into consideration. For example, do they receive the same acoustic information from fish? Is it possible to use the equations developed for dual-beam systems in split-beam systems without obtaining different results about biomass and density?

Previous studies have analysed the acoustic results obtained using dual and split-beam systems. Traynor and Ehrenberg (1990) registered TS values coming from a calibration sphere using both systems. They found that the values obtained with split-beam systems presented fewer variations than those obtained using dual-beam systems. Subsequently, Ehrenberg and Torkelson (1996) used theoretical results and compared the effect that noise had on both systems. They found that split-beam systems were the least affected by the increase of sound and, therefore, were more appropriate to study noisy environments. These works were performed using theoretical models and targets whose sound was previously known. They answer the first question and show that both systems (dual-beam and split-beam) receive the acoustic information from targets in different ways. Thus, both systems likely present variations in fish detection.

In article II (**Horizontal target strength of *Cyprinus carpio* using 200 kHz and 430 kHz split-beam systems**), the horizontal conversion equations for *Cyprinus carpio* (common carp) have been developed using two different frequencies. The common carp is also a very important species in fish communities in European freshwater systems. It is native to Asia and it can be found everywhere in the world, except for the Middle East and the Poles (Kottelat and Freyhof, 2007). This species significantly contributes to the total biomass of freshwater systems due to its large size and abundance. Therefore, there is no doubt that its acoustic study will improve the results of the acoustic surveys of these ecosystems.

Besides creating these conversion equations, this article also focuses on the possible variations that may arise when the equations developed for dual-beam systems are used in studies where split-beam systems are employed. To that end, the horizontal conversion equations developed for common carp with two split-beam systems operating at 200 and 430 kHz have been compared with those created by Kubecka and Duncan (1998) for the same species and frequencies using dual-beam systems.

Another question considered by researchers when selecting the acoustic system is which frequency should be used. On the one hand, the quality of the biomass and density estimates depends on the choice of an appropriate conversion equation and, on the other hand, on the selection of a proper frequency.

Compared to low frequencies, high frequencies render better system resolution, although the results obtained are not good when fish density is high or when there are large amounts of noise (Simmonds and MacLennan, 2005). Degan and Wilson (1995) recommend using frequencies between 120 and 200 kHz for fish detection, although higher frequencies have been applied in other studies to analyse the sound of fish or to estimate the density of fish populations (Kubecka *et al.*, 1994; Beauchamp *et al.*, 1997; Kubecka and Duncan, 1998). In horizontal hydroacoustics, the selected frequency does not only have to allow recognising individual fish, but it also has to accurately position the returned echoes since the calculation of the fish's swimming angles is fundamental when interpreting acoustic data. There are not many established comparisons between frequencies (Wanzenböck *et al.*, 2003; Guillard *et al.*, 2004; Godlewska *et al.*, 2009). Furthermore, in horizontal hydroacoustics, there are no studies comparing the capability of different frequencies to position echoes. These studies are necessary because, on the one hand, they develop our knowledge about the functioning of different frequencies and, on the other hand, they provide tools to determine which frequency is the most appropriate for a particular study.

In light of these needs, article II analyses the position estimates obtained at 200 and 430 kHz using tracks of simultaneously ensonified fish. This analysis aims to determine at which frequency the position of the tracks of moving fish in shallow systems is most accurately estimated.

The usable distance of sampling is another important factor to be taken into consideration when analysing data obtained by means of horizontal hydroacoustics. First of all, the reverberation of the surface and the bottom limits the usable range for the analysis when studying superficial and shallow systems (Mulligan, 2000). The parameter used to quantify this effect is the signal to noise ratio (SNR), i.e. the ratio between the noise and the signal of the fish. Consequently, at a given distance from the transducer, the effect of the reverberations can be so large that the signal of the fish cannot be distinguished (low SNR), which would limit the maximum distance for the analysis.

Secondly, there is another effect that limits the minimum distance for the analysis, i.e. the distance that has to be maintained between the transducer and the fish so that the collected data are correct. Theoretically, TS values should not be measured within the area known as near-field (Simmonds and MacLennan, 2005). The near-field is adjacent to the fish's surface. Within this area, the sound experiences many oscillations, which in turn renders TS measurements unstable (Dawson *et al.*, 2000). The near-field is calculated based on fish length and is therefore directly related to this parameter. For example, a 400 mm fish has a near-field of approximately 5.5 m, which means that the theoretical minimum distance to be maintained between the fish and the transducer should be 5.5 m. This considerably limits the available volume for the sampling (Figure 4).

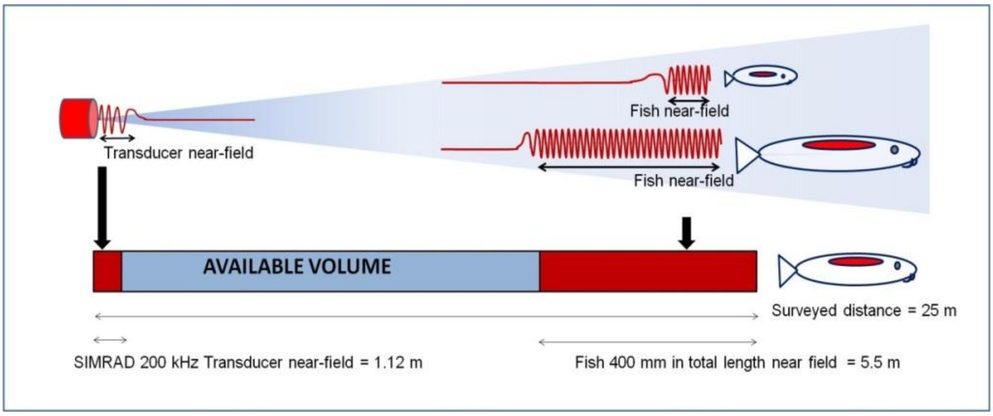

Figure 4. Effect of the near-field. The upper graph shows the near-fields of the transducer and two fish with different sizes. The lower graph represents the ensonified volume (black rectangle), the space occupied by the near-fields of the transducer and a fish with a given length (highlighted in red) and the theoretical volume available for the analysis (highlighted in blue).

The last question regarding distance is related to the ensonification of large fish located close to the transducer. The majority of the acoustic signal emitted by the transducer is scattered conically. Therefore, large fish located close to the transducer may be only partially ensonified and their TS may be different from that obtained by the same fish from a greater distance where they are entirely ensonified. These limitations (maximum and minimum distance from the transducer) may sometimes invalidate the information collected from hydroacoustic samplings conducted with horizontal orientations.

Article III (**Do close range measurements affect the target strength (TS) of fish in horizontal beaming hydroacoustics?**) aims to resolve some of the questions regarding distance. Its main objective is to determine the range where TS values are stable within the first metres of ensonification (6, 9 and 12 m) when performing horizontal samplings.

The results obtained in this doctoral thesis are intended to improve the use of hydroacoustic techniques in horizontal orientations and, therefore, their usefulness as a tool to study fish populations in epicontinental aquatic ecosystems. The new developed equations will help improve the results of studies dealing with species that are dominant components in fish communities, such as in most reservoirs on the Iberian Peninsula. A deeper understanding of sound behaviour in the studied situations will help establish scientific criteria to select the most appropriate tools and systems to be used depending on the features of the given ecosystem. Furthermore, it will lead to a better interpretation of the acoustic data obtained by means of different systems. Finally, the study of sound behaviour depending on distance confirms the usefulness and applicability of hydroacoustics to analyse fish communities in shallow systems. It also provides useful information to determine the most appropriate distance to collect and analyse data.

RESULTS

This thesis consists of three articles, two of which (articles I and III) have already been published. The remaining article (article II) has also been sent to an international scientific journal and it is on the way to be published.

ARTICLE I

Horizontal target strength of *Luciobarbus sp.* in *ex situ* experiments: testing differences by aspect angle, pulse length and beam position.

Rodríguez-Sánchez, V., Encina-Encina, L., Rodríguez-Ruiz, A., Sánchez-Carmona, R. (2015). Fisheries Research, **164**, 214–222.

This study provides TS-length horizontal conversion equations for barbel, a very important species in the European fish communities for which there had been no acoustic information thus far. Furthermore, we have studied the effect that the selection of different pulse lengths has on the energy backscattered from fish. We did not find any difference in the sound backscattered from the studied fish even when using different pulse lengths. We have also dealt with two of the most frequent problems associated with the development of conversion equations, i.e. the estimation of the fish's swimming angle when using free-swimming fish and the high amount of time required to form the equations. Regarding the problem of estimation, a new method has been proposed. This method includes all of the sound information backscattered from moving fish and it accurately represents their orientation with respect to the transducer plane. With regard to the problem of the time, we have studied the backscattered TS in different positions in the acoustic beam. The results allow for an extension of the usable area to acquire data and for a reduction in the time required to develop the conversion equations.

ARTICLE II

Horizontal target strength of *Cyprinus carpio* using 200 kHz and 430 kHz split-beam systems.

Rodríguez-Sánchez, V., Encina-Encina, L., Rodríguez-Ruiz, A., Monteoliva, A., Sánchez-Carmona, R.

In this study, the TS-length conversion equations for the species *Cyprinus carpio* (common carp) have been developed using two split-beam systems operating at different frequencies (200 and 430 kHz). On the one hand, the possible differences associated with frequency have been studied, both in the perception of signals from fish and between the conversion equations. On the other hand, we have studied the differences between the new conversion equations generated in this study and those developed for the same species using a dual-beam system. The results showed that the differences are more pronounced when comparing systems with different beams (dual-beam *vs.* split-beam) than when comparing different frequencies (200 and 430 kHz). We have also studied the potential displayed by both frequencies to detect and position the fish tracks. The results reveal that differences exist in fish detection depending on the frequency that is used. According to our results, the 200 kHz frequency renders better results than that of 430 kHz when studying an individual fish and when determining its position in superficial or shallow waters.

ARTICLE III

Do close range measurements affect the target strength (TS) of fish in horizontal beaming hydroacoustics?

Rodríguez-Sánchez, V., Encina-Encina, L., Rodríguez-Ruiz, A., Sánchez-Carmona, R. (2015). Article in press: Fisheries Research (2015), http://dx.doi.org/10.1016/j.fishres.2015.03.020

This article deals with the efficiency of horizontal hydroacoustics samplings conducted at close range. Both the near-field effects and the behaviour of TS with regard to distance have been studied. We have found that the theoretical near-field may be overestimated since it is calculated based on the fish length. Instead, it may be calculated based on the length of the swim bladder, which is the organ responsible for most of the energy backscattered from fish. Our results show that a recalculation of the near-field based on swim bladder length reduces the theoretical distance that is usually recommended to be avoided when acquiring acoustic data. This increases the volume available for acoustic analysis without causing variations in TS. Furthermore, our results demonstrate that TS values of fish remain stable within the first 12 m of the sampling. This proves that hydroacoustic data recorded with horizontal orientations are stable within the distances that are most commonly used. It also emphasises the benefits of using this method when shallow water systems are to be studied.

Note to readers:

The author of this paper understands that this introduction may appear to be a bit long for a specialised audience. This didactic work is intended to reach readers who are not experts in the field and are interested in the study of fish by means of hydroacoustic methods. In order to help understand the text, certain terms have been defined, some relevant effects have been clarified and the acoustic theory has been explained in greater detail.

This thesis consists of three articles, two of which (articles I and III) have already been published. The remaining article (article II) has also been sent to an international scientific journal and it is on the way to be published.

CHAPTER 1:

Horizontal target strength of *Luciobarbus sp.* in *ex situ* experiments: testing differences by aspect angle, pulse length and beam position.

Rodríguez-Sánchez, V., Encina-Encina, L., Rodríguez-Ruiz, A., Sánchez-Carmona, R. (2015). Fisheries Research, **164**, 214–222.

Fisheries Research 164 (2015) 214–222

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Horizontal target strength of *Luciobarbus* sp. in ex situ experiments: Testing differences by aspect angle, pulse length and beam position

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ARTICLE INFO

Article history:
Received 2 May 2014
Received in revised form
24 November 2014
Accepted 26 November 2014
Handling Editor George A. Rose
Available online 30 December 2014

Keywords:
Luciobarbus sp.
Hydroacoustics
Target strength
Side aspect
Pulse duration

ABSTRACT

Horizontal applications of hydroacoustics are used to estimate the density and biomass of fish populations in shallow aquatic ecosystems. In order to calculate fish biomass and density, it is necessary to know the relationship between the biological parameters, such as the length or weight of the studied specimens and the associated target strength (TS). This study presents the results of an ex situ experiment performed on free-swimming specimens of *Luciobarbus* sp., one of the most diversified and widely distributed genus of the family Cyprinidae. The study was performed using a SIMRAD EK60 split beam echo sounder at 200 kHz. TS–length (TS–TL) and TS–weight (TS–W) relationships were derived in the three main orientations: lateral, oblique and head–tail. This study uses a new method in order to calculate the fish aspect angle. This method takes all backscattered fish energy into account and it summarizes the fish movement through regression lines. The TS–TL relationship for the barbel lateral aspect was $TS_{lateral} = 25.03 \log TL - 99.4$ and its results were different from those obtained with other previously published general equations. Thus, the use of this specific equation is recommended in order to obtain accurate estimates of density and biomass in aquatic systems where the barbel is an important component of the fish community. Moreover, the effect of pulse duration on the estimated TS was studied. The results showed no differences between the mean TS obtained by using 0.128 ms or 0.256 ms of pulse length. Additionally, the influence that the location of the track in the beam has on mean TS was also studied. The results showed that this location does not have a significant influence on fish TS within the first −5 dB (one-way) of the beam pattern. This result provides us with a powerful tool to increase the amount of data in TS studies with free-swimming fish where the acquisition process is difficult.

1. Introduction

Hydroacoustics is a widespread technique used to estimate the size of fish stocks and their related biological parameters, as well as to map their spatial distribution (Encina et al., 2008; Emmrich et al., 2010) or their behavioural and trophic relationships (Paramo et al., 2003; Vehanen et al., 2005; Draštík et al., 2009). The use of this remote observational technique is increasingly being considered as an option and it represents an effective alternative to conventional survey techniques in terms of quantitative measurements of fish density or biomass (Kubecka et al., 2012). Among the advantages of acoustic methods, we can point out the fact that it is a non-invasive method that allows for exploration of large areas in a relatively short period of time with a high spatial and temporal resolution (Godlewska and Jelonek, 2006).

In order to obtain the absolute fish density, it is necessary to know the fish acoustic backscattering cross section or its logarithm target strength (TS dB re 1 m^2) (Hartman and Nagy, 2005). This feature is strongly related to the fish length and it is influenced, among other factors, by changes in the angle of the fish related to the sound beam (Love, 1977; Hazen and Horne, 2003; Simmonds and MacLennan, 2005; Jech, 2011). Particularly, in shallow fresh-water systems, lateral aspect TS is important for acoustic surveys of fish and it is necessary to study the changes in TS along different orientations (Pedersen et al., 2009). In the last fifteen years, previous research has described the relationship between TS and the real size of individual species, mostly in studies where transducers were aimed vertically, i.e. with the beam axis perpendicular to the water surface (Nielsen and Lundgren, 1999; Gauthier and Rose, 2001; Axelsen et al., 2003; Ona, 2003; Knudsen et al., 2004; Didrikas and Hansson, 2004; Borisenko et al., 2006; Reine et al., 2010).

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http://dx.doi.org/10.1016/j.fishres.2014.11.020
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Much fewer studies have been carried out in horizontal orientation (i.e. with the beam axis parallel to the water surface) (Burwen and Fleischman, 1998; Lilja et al., 2000; Frouzova and Kubecka, 2004; Frouzova et al., 2005; Boswell and Wilson, 2008) and most of them were performed using anaesthetized or immobilized fish, which leads to differences on TS measurements (McClatchie et al., 1996). Therefore, this study uses free-swimming fish, which makes the research more difficult and highly time-consuming, but it may provide results closer to those found in natural environments.

The genus *Barbus* (Bailly, 2014), one of the most diversified of the family Cyprinidae, is widely distributed across the Old World (Africa, Europe and Asia), where it is the dominant component in the cyprinid fauna (Callejas and Ochando, 2002). In Europe, all species of *barbus* and some species from North Africa and West Asia are included in two *barbus* subgenera: *Barbus* and *Luciobarbus* (Wang et al., 2013). In the Iberian Peninsula, the subgenus *Luciobarbus* is one of the most important of all freshwater fauna and it has the highest specific diversity among all fish genera (Encina et al., 2006). Despite its location and wide distribution over three continents (Buonerba et al., 2010), there is no information about its acoustical properties, since it has never been studied. The subgenus *Luciobarbus* was selected because of its importance in the European fish community and because of the lack of information about its scattering properties, which is fundamental for biomass calculations.

The aim of this study was to establish the relationship between the TS and body length from their lateral, oblique and head–tail orientations for *Luciobarbus* sp., since experience has shown that such relationships are crucial in determining the fish size from acoustic records, especially in horizontal applications (Kubecka and Duncan, 1998). In order to improve the accuracy of acoustic-based length and abundance estimates, we have checked some factors that may influence TS. First, we have studied the influence of different pulse lengths on TS results, because pulse length is a data acquisition setting and cannot be modified after survey. Therefore, its study could provide us with tools to select the proper setting for each survey. In addition, the effect of the location of the target with respect to the beam axis has also been studied (Medwin and Clay, 1998). Thus far, the use of echoes from the theoretical central part of the acoustic beam has been recommended in studies where TS accuracy is needed (Henderson et al., 2007). We have measured the backscatter from fish located inside and outside of the −3 dB half-power points or half-intensity angle of the beam. To this end, we have increased the beam pattern to −5 dB and we have compared the TS produced by tracks located in different positions across the studied beam in order to understand how the position of the target in the beam may influence TS. We consider this an interesting comparison because, if there were no differences between TS results, we could increase the number of valid echoes without affecting mean TS measurements and data would be easier to obtain. Therefore, we have used ex situ target-tracking measurements to research how orientation and pulse length combined with the position of the tracks in the acoustic beam may influence fish TS.

2. Material and methods

2.1. Fish collection and preparation

Seven groups of Andalusian barbel (*Luciobarbus sclateri*) and Iberian barbel (*Luciobarbus bocagei*) with different sizes were used in the experiment. Small fish (<10 cm) were collected from a river by electrofishing and large fish were brought from the fish farmer “Vegas del Guadiana” in Badajoz. All fish were brought to the laboratory and were anaesthetised with clove oil to prevent stress and injuries during the transport (García-Gómez et al., 2002). All fish were put in quarantine for ten days to ensure their well-being. Feeding was stopped during the experiment. Before the recordings, fish total length (TL), fork length (FL) and standard length (SL) were measured in millimetres and weight was measured (W) in grams.

Table 1
Features of fish groups: ID, fish size class, number (N) and mean total length per group in cm (Mean TL).

ID	Size class	N	Mean TL (cm)
Group 1	1	5	6.8
Group 2	1	3	7.6
Group 3	1	3	9.2

After the measurements, the fish were left for 48 h in a specially designed cage in order to perform the subsequent acoustical recordings at each of the studied pulse lengths. Underwater video recordings were collected throughout the experiment in order to observe specimen behaviour (Boswell and Wilson, 2008) and to be used as supporting media in the data acquisition process. Video data showed that fish were assuming natural swimming behaviour during the experiments without avoiding the acoustic beam.

Small fish (<10 cm) were gathered together in three groups to ensure that signals were recorded (Table 1). The first group was comprised of 5 individuals with a mean length of 6.8 cm. The second and the third group were comprised of 3 specimens with mean lengths of 7.6 and 9.2 cm, respectively (Table 1). For these three groups of small fish, underwater video recordings were used to check that the selected tracks belonged to individual fish. These recordings allowed us to avoid that overlapping echoes from multiple targets were falsely accepted as valid echoes from the same track. Large fish (>10 cm) were placed individually and a total of 25 individual fish with total lengths between 10 and 70 cm were ensonified.

After the recordings, fish were sorted into 10-cm bins. There were a total of seven size classes, each one comprising of at least three individuals.

2.2. Experimental setup

The cage created to hold the fish was a cube with an edge length of 1.5 m made in PVC pipe. The frame had holes in order to let water pass through it and to avoid undesirable air bubbles (Hartman and Nagy, 2005). The cage was covered by means of a mesh. The back and frontal walls of the mesh were made in monofilament netting (5 mm knot to knot) in order to let the sound pass through the cage and to avoid undesirable noise. In order to further evaluate the acoustic transparency of the cage, a calibration sphere (TS = −45 dB) was used. We studied three different positions with respect to the transducer: in front of the cage (from 5.5 to 6 m), in the middle of the cage (between 6.5 and 7 m) and behind the cage (from 7.5 to 8 m). In each case, a mean deviation smaller than 0.5 dB was found in the expected TS and no significant echo returns were recorded due to the cage. The frame was located in front of the transducer at a distance of 6 m, where the diameter of the acoustic beam cross section allowed for a complete ensonification of the largest fish and avoided the near-field effects of the applied transducer (i.e. 0.56-m) (Tichy et al., 2003; Knudsen et al., 2004; Handegard, 2007).

2.3. Data collection

A Simrad EK60 scientific echo sounder (Simrad Kongsberg Maritime AS, Horten, Norway) equipped with a 200 kHz circular split-beam transducer (ES200-7C) was used for all TS measurements. The transducer was mounted at a water depth of 2.5 m. It was horizontally oriented (i.e. with the beam axis parallel to the water surface) on a stainless steel bar held by a fixed expanded

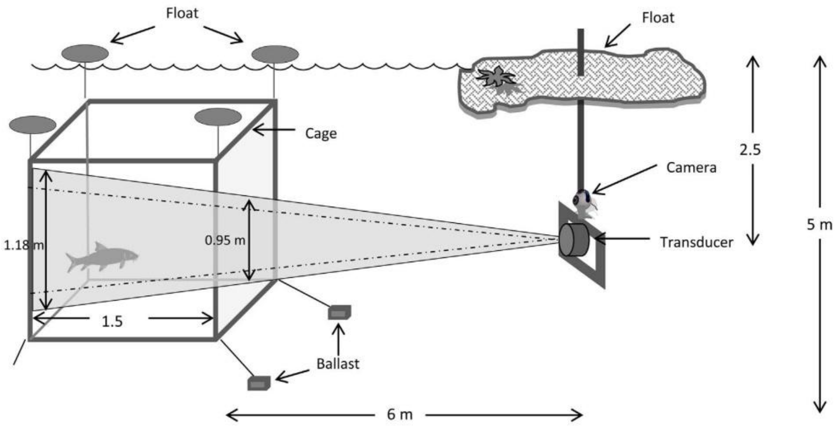

Fig. 1. Schematic view of the experimental setup. The transducer is mounted in front of the cage with a video camera on it. The cage is fixed to the bottom by four ballasts and suspended by four floats.

polystyrene (EPS) sheet in order to maintain the buoyancy (Fig. 1). The acoustic unit was calibrated with a 13.7-mm copper sphere (TS = −45 dB) according to the standard calibration method (Foote et al., 1987). The calibration was performed separately for each pulse length (0.128 and 0.256 ms). The echo sounder was set to store data at a ping rate of 10 ping/s. Data were stored on a PC and later processed with the Sonar5-Pro v.6.0.1. analysis software (Balk and Lindem, 2011).

In the software Sonar5-Pro, the TS threshold for data analysis was set to −70 dB in order to eliminate noise. TS corrected for angular location in the beam (TS, dB re 1 m^2) were used for the analyses. The condition used to distinguish individual targets was a minimum and maximum echo length of 0.80 and 1.6 times the transmitted pulse length. Maximum gain compensation was −5 dB (one-way) and maximum phase deviation was 8. We applied this setting in order to ensure that we obtained as many detections as possible from the recorded fish.

2.4. Acoustic processing

Tracks were selected manually using the manual tracking tool from the analysis menu in Sonar5-Pro (Balk and Lindem, 2011). In order to be included in a track, a target could not be separated from the previous one by more than three pings (Henderson et al., 2007). Each track needed to contain at least five echoes to be considered valid. In order to obtain a better calculation of the aspect angle, it was important to ensure that the selected tracks showed fish swimming in a straight path without changes in the swimming direction. The tracks located close to the edges of the cage and those with low quality were rejected. Thus, only high quality linear swimming tracks produced by a single fish were accepted.

In order to compare tracks located in different positions across the studied beam pattern (−5 dB one-way of gain compensation), they were classified into two categories depending on their position in the beam. The Athwart (*X*-deg) and Along (*Y*-deg) positions given by the post-processing software were used to sort the tracks into central tracks (CT), comprising tracks within the first 2.5° from the acoustic axis, and out tracks (OT), with tracks located 2.5 to 4.5° away from the acoustic axis.

In order to calculate the aspects of the fish tracks, we realized it was important to take into account the complete movement of the fish, including all echoes produced. Consequently, an alternative method was developed and implemented in Sonar5-Pro to calculate tracks' angles. As Fig. 2 shows, we have removed the *Y*-axis from our calculations. The track of a fish has been projected on the *XZ* plane removing the *Y* component of the track vector. The regression line of all *XZ* positions has been calculated as follows:

$$z = mx + n, \tag{1}$$

where m is the slope of the regression and n is the constant.

Then, the aspect angle of the selected fish track with respect to the *XY* plane of the transducer was obtained using the next formula:

$$\vartheta = \arctan m + 90^o,$$

where m is the slope of the calculated regression.

In order to relate the angle to the acoustic axis, 90° were added. The aspect where the axis of the fish was perpendicular to the acoustic axis was fixed at 90° and named "side aspect".

The mean TS of each track was calculated as the mean value of all echoes in a fish track. Mean TS was calculated in the linear domain as follows:

$$\text{Mean} \quad \text{TS} = \bar{\text{TS}} = 10 \log \left(\frac{1}{N} \sum_{i=1}^{N} 10^{\text{TSi}_i/10} \right) \tag{2}$$

Tracks were classified in groups of 2° according to their aspect angle (0–2°, 2–4°, 4–8°, etc.) and the mean TS was calculated for each group. Subsequently, each group was gathered into bins of 10-degrees. The mean TS and its 95% confidence interval (95% CI) were calculated for each aspect category in order to allow for direct comparisons among factors within the model. Consequently, three angular regions were established to describe the TS responses by fish angle with respect to the acoustic beam: head–tail orientation (comprising angles between 0–20° and 160–180°), oblique orientation (with angles between 20–70° and 120–160°) and lateral orientation (70–120°) (Boswell and Wilson, 2008).

2.5. Statistical analyses

Acoustic data collected from free-swimming fish were statistically analysed by SPSS 20.0. (Armonk, NY). Before conducting an

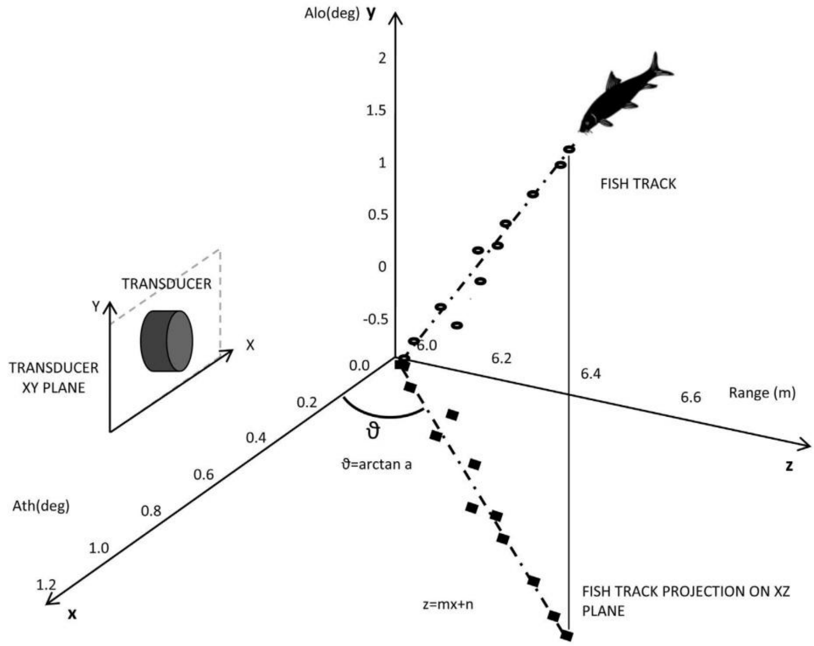

Fig. 2. Explanatory scheme about how we calculated track angles from free-swimming fish.

ANCOVA, the homogeneity of regression (slope) assumption was first tested. The test evaluated the interaction between the covariate (length) and the factor (position of the track in the beam, pulse length and aspect angle) in the prediction of the dependent variable (TS). A one-way analysis of covariance (ANCOVA, $\alpha = 0.05$) was conducted to test the effect of the studied factors on the variability of the mean TS, with the total length as the covariate. In the comparison conducted to quantify the influence of the position of the track in the beam, data were separately compared by pulse length and orientation in order to avoid possible effects on the dependent variable. In order to study the variation of TS with aspect angle, we pooled all data.

The proportion of the total variability explained by the individual factors (omega squared) was calculated as the sum of squares accounted for the independent variable:

$$\omega^2 = \frac{SS_i - (K-1)MS_{error}}{SS_T + MS_{error}} \quad (3)$$

where SS_i is the Type III sum of squares of the independent factor; MS_{error} is the mean square of the error; SS_T is the Type III sum of squares of the corrected total value; and K is the degree of freedom of the corrected model (IBM Corp., 2011).

Linear regression models were used to describe the relationship between TS and length or weight by orientation. The TS values measured for each orientation were regressed against weight as well as the various lengths (standard, fork and total) used in fish research. The relationship was modelled with the equation $Y = a \log X + b$, where Y is the target strength (TS) in dB, X is the length or weight of the fish and a, b are regression constants.

3. Results

The results showed no significant differences among the mean TS values backscattered from targets located at different positions in the beam $F_{2.147} = 0.914$, $p = 0.341$ for pulse length 0.128 ms and $F_{2.189} = 0.306$, $p = 0.581$ for pulse length 0.256 ms. The homogeneity of regression assumption indicated that the relationship between the covariate and the dependent variable TS did not differ significantly as a function of the position of the target in the beam, $F_{3.146} = 2.211$, $p = 0.139$ for 0.128 ms and $F_{3.188} = 0.291$, $p = 0.407$ for 0.256 ms. Moreover, each orientation showed similar TS values regardless of the location of the target (ANCOVA; $F_{1.73} = 0.087$; $p = 0.769$ for head–tail orientations; $F_{1.173} = 0.023$; $p = 0.879$ for oblique orientations and $F_{1.113} = 1.542$; $p = 0.217$ for lateral orientations). Then, the mean TS obtained for tracks located inside the central part of the beam (CT: 0–2.5° from the acoustic axis) did not differ from the mean TS obtained for tracks located out of the central part of the beam pattern (OT: 2.5–4.5° from the acoustic axis). Therefore, TS values were combined for further analysis.

The mean TS responses averaged over 10° of aspect angle were plotted separately by pulse duration (Fig. 3). With a pulse length of 0.128 ms, the mean TS obtained was −41.78 dB (St. Dev. = 6.24) and the resulting mean TS for the 0.256 ms pulse length was −41.29 dB (St. Dev. = 5.30), i.e. less than one decibel of difference. The results of the analysis of covariance showed that the independent variable (pulse length) did not have a significant effect on the TS–log TL relationship, and the slopes of the regression lines were statistically equal, $F_{3.338} = 1.600$, $p = 0.207$. Additionally, no significant differences were found in the mean TS obtained by each pulse length (ANCOVA; $F_{2.331} = 0.645$, $p = 0.423$), regardless of fish orientation.

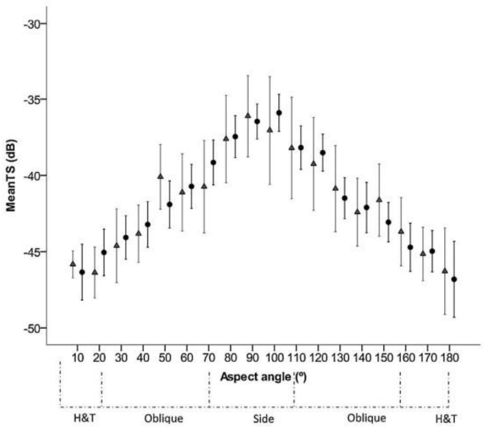

Fig. 3. *Luciobarbus* sp. TS (dB) and confidence interval (95%) distribution averaged over 10° swimming-angle degrees separated by pulse duration. Orientation labels are below the angular position and they represent the regions used in data analysis to describe the effects of fish horizontal orientation on TS. Dots correspond to TS values obtained by a pulse length of 0.128 ms and triangles correspond to 0.256 ms.

The mean TS was significantly influenced by the aspect angle (ANCOVA; $F_{2,338} = 315.266$, $p < 0.01$). Changes in the fish swimming direction resulted in mean TS differences. Nevertheless, only 28% of the total TS variability (ω^2) was explained by the different aspects presented by fish while they were swimming in front of the transducer. The TS increased as fish were swimming from a head–tail position to a lateral position with respect to the plane of the transducer in all the studied sizes and the maximum TS occurred at lateral incidence (around 90°) (Fig. 4). The average line showed a difference of 9 dB between the maximum and the minimum TS.

TS–TL regressions were not the same among orientations, although they maintained the same relationship in terms of slope, regardless of orientation (ANCOVA; $F_{2,336} = 1.962$, $p = 0.142$). Regression slopes produced almost parallel regression lines, although the intercepts changed from 5 to 10 dB and the TS values were different for the same length depending on the orientation (Fig. 5). The variation of TS overall average aspects ranged from 6 to 10 dB over all fish deployed at a given length. The maximum TS was obtained with lateral orientations and these values were substantially higher than those obtained for head–tail orientations in all size classes.

The fitted models of pooled data calculated for TS and the different lengths and weights by orientation are listed in Table 2. The coefficients of the regressions calculated for the different lengths ranged from 21.78 to 25.03 for values *a*. The coefficients *b* ranged from −100.86 to −94.36 dB. All regressions were significant (ANOVA; $p < 0.001$) and the equations showed a high correlation (R^2) between the dependent variable TS and the related independent variables.

4. Discussion

The fish's backscattered echo is directly related to its length or its weight. Therefore, the choice of an appropriate method and a suitable conversion equation is fundamental in order to obtain accurate estimates of fish biomass. This need is highlighted by the comparison carried out between the results obtained from previously published TS–length equations for horizontal orientation (Table 3) and those obtained in this study (Fig. 6). As a result, we have confirmed that, in the case of barbel, depending on the equation used, TS estimates present certain deviations that usually underestimate fish size. The relationships that Frouzova and Kubecka (2004) established for river perch provided length

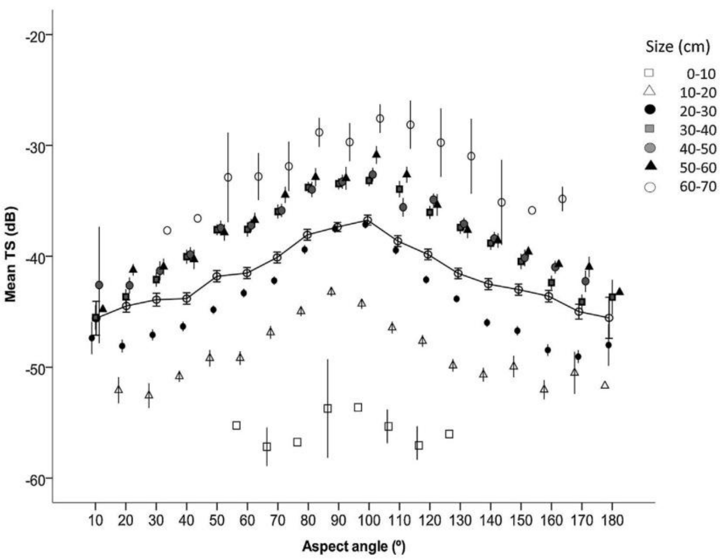

Fig. 4. Estimated mean TS for each size averaged over 10° swimming-angle degrees and confidence interval (95%). The black solid line corresponds to the mean TS from all size classes.

Table 2
Linear regressions of horizontal target strength (TS) in dB vs. fish length (mm) and weight (g) by orientation.

Orientation	Variable	*N*	*a*	Std. E.	*b*	Std. E.	R^2	*p*
H/T	TL	11	22.46	1.99	−100.86	5.03	0.927	<0.001
Oblique	TL	12	22.52	1.18	−97.63	2.94	0.970	<0.001
Lateral	TL	12	25.03	2.07	−99.44	5.14	0.929	<0.001
H/T	SL	11	22.46	1.82	−100.00	4.54	0.938	<0.001
Oblique	SL	12	22.46	1.22	−96.61	2.98	0.968	<0.001
Lateral	SL	12	24.91	2.16	−98.17	5.27	0.923	<0.001
H/T	FL	11	21.90	1.75	−98.03	4.32	0.939	<0.001
Oblique	FL	12	21.78	1.15	−94.36	2.78	0.970	<0.001
Lateral	FL	12	24.17	2.05	−95.71	4.96	0.926	<0.001
H/T	W	11	7.63	0.67	−63.92	1.79	0.927	<0.001
Oblique	W	12	7.24	0.39	−59.46	1.01	0.968	<0.001
Lateral	W	12	8.12	0.57	−57.19	1.47	0.947	<0.001

Table 3
Horizontal TS–length relationships, TS = *a* log L + *b* derived from some previous published studies for freshwater fish.

Specie	Length	*a*	*b*	Freq (kHz)	Orientation	Author
Chinook salmon	TL (cm)	17.8	−56.8	200	All aspects	Burwen and Fleischman (1998)
Pool of freshwater species	SL (mm)	23.5	−84.6	200	lateral	Kubecka and Duncan (1998)
Alewives of perch	TL (mm)	19.8	−85.8	120	lateral	Frouzova and Kubecka (2004)
Pool of freshwater species	TL (mm)	24.7	−89.6	120	lateral	Frouzova et al. (2005)

values more similar to ours because of the similarities between studies, although the size range studied was narrower and it only comprised the larval stage. Burwen and Fleischman (1998) developed an in situ experiment with salmon and their results were more different, probably due to the great differences that salmonid presents with respect to cyprinid and the size range used in their study, where the average length was greater than that found in our largest size class. The pooled-species regressions obtained by Kubecka and Duncan (1998) and Frouzova et al. (2005) were similar to our relationship in terms of slope, but TS–length regressions underestimated the length of barbel. All these differences were expected due to the fact that regressions were made on different species and because of the variable nature of TS, which may change even for the same fish (Rose and Porter, 1996; Rudstam et al., 2003; Godlewska, 2004; Handegard, 2007, Handegard et al., 2009).

The TS–length and weight relationships proposed for horizontal beaming for *Luciobarbus* sp. contribute to the amount of species

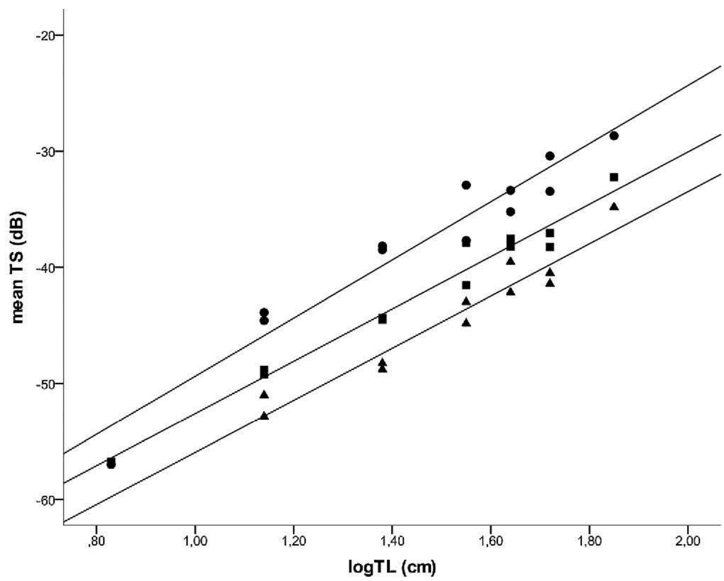

Fig. 5. TS–total length relationships derived from deployed fish separated in different lines by orientation. Triangles indicate head–tail orientation, squares indicate oblique orientations and dots indicate lateral orientations.

that are acoustically described using horizontal beaming. These relationships could be useful because, thus far, there was no acoustic information about this species, even though it is an important component of many freshwater fish communities.

During this experiment, we found several difficulties when trying to obtain quality tracks from the area recommended for developing conversion equations (i.e. the −3 dB half-power points of the beam pattern). Using live fish made the data acquisition process more time-consuming since the free-swimming fish needed to cross the beam within a given area. The equations for horizontal orientation developed to date limit the available space to the central part of the main beam (Kubecka et al., 2009; Lilja et al., 2000; Frouzova et al., 2005). In these experiments, data acquisition was not a problem since the authors used immobilized fish that were placed in front of the acoustic beam in the desired position. In the cases where the data acquisition process poses a problem, other authors recommend the use of a larger beam compensation to increase the number of valid detections (Rudstam and Sullivan, 2014). Following this recommendation, we studied the behaviour of TS beyond these −3 dB one way of beam width, increasing the beam compensation to −5 dB. Nevertheless, given that the TS tends to decrease as it moves away from the acoustic centre, we previously checked that there were no differences in the mean TS between tracks located in different positions in the acoustic beam. This allowed us to use all the tracks available in the studied beam pattern (−5 dB compensated or 9° of beam width) to develop the relationships. This void effect on TS could probably be enhanced by a good calibration process that minimizes possible variations in the detected energy along the cross beam section. This is why good calibrations are important for these types of studies. In our case, this slight increase in the volume available for the analysis involved a 25% increase in the number of valid tracks. Thus, we found this option very interesting because it considerably reduces the time required for data acquisition, which moderates one of the difficulties faced by the investigator when developing this kind of experiment.

Nevertheless, this increase in the available area must be carefully taken into account since its validity has only been proven at the studied distances, i.e. at a close range. At these distances, there is less sound dispersion and the energy loss may not be shown in the received TS (Simmonds and MacLennan, 2005). Besides, the stability of the TS value may also be favoured by the controlled conditions under which this experiment was carried out. Therefore, it is not recommended to use this method for density or biomass studies carried out in natural systems because the analysis could include sounds that are distant from the acoustic centre which present an important drop in the received echo signal. Their inclusion in the analysis could entail deviations in the results.

Since the orientation of the fish body with respect to the sound beam is one of the factors proven to have the most significant effect on the TS, this study provides the horizontal TS conversion equations for barbel in the three main orientations: head–tail, oblique and lateral. In order to develop these equations, we have applied a new method to calculate the aspect angle. The need to implement this new method arose when we realized that there was an imbalance between the track aspect provided by the post-processing program and the aspect presented by the fish in the echograms and simultaneous video recordings.

Sonar5-Pro allows the calculation of the aspect of any track by means of two different methods. In the first method, the aspects between the consecutive echoes of the track are calculated in order to later calculate the average of all aspects obtained. In the second

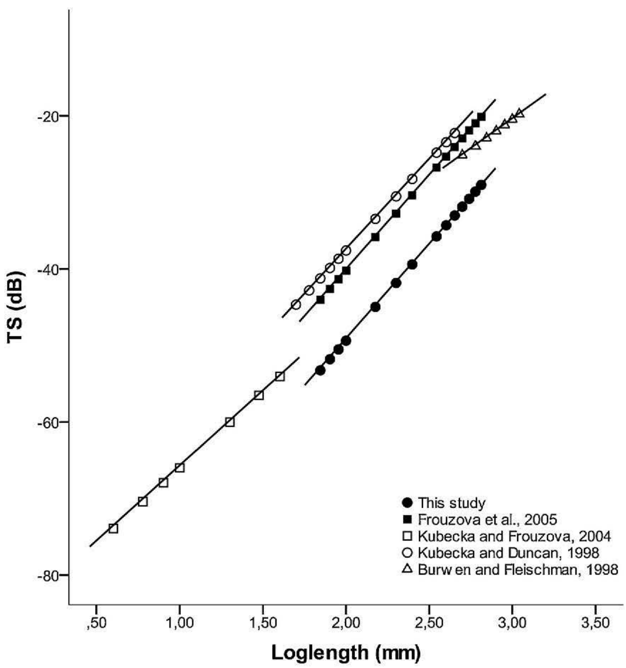

Fig. 6. Regressions TS–length for pooled data from barbel (6–70 cm), and TS–length equations proposed by different authors.

method, the difference in the position between the first and the last echo of the track is calculated and the aspect is calculated from that difference. As previously stated, none of them provided an angle that reflected fish orientation. However, the method developed in this study provided a swimming angle in accordance with the angles shown in the video recordings. Based on the results obtained, we consider regression to be the procedure that better summarizes fish track information because, when regression lines of the fish trajectories are calculated, the possible biases caused by the natural movement of fish are moderated. In addition, this kind of calculation takes all the backscattered fish energy into consideration, which is closer to the sound that we could find when doing a survey. This new method has been included in the new version of Sonar5-Pro.

In line with prior studies (Kubecka and Duncan, 1998; Lilja et al., 2000; Rudstam et al., 2003; Frouzova et al., 2005; Boswell and Wilson, 2008; Gurshin, 2012), lateral orientations gave TS values significantly greater than oblique or head–tail orientations. This is mainly due to the effect of the increased exposure of the surface body area as fish swam in a more perpendicular manner with respect to the acoustic axis together with the increase of the exposure of the swim bladder (Boswell and Wilson, 2008). Although high variations on TS values were registered due to the different swimming angles (Kubecka and Duncan, 1998; Torgensen and Kaartvedt, 2001), the differences between the maximum TS (lateral orientation) and the minimum TS (head–tail orientations) were found within the previous published range of variability and they were not greater than 10 dB (Godlewska et al., 2012; Burwen and Fleischman, 1998). Boswell and Wilson (2008) found a variation on TS for bay anchovy and gulf menhaden lower than 3.5 dB. This slight variation was probably due to the narrow size range of the fish available during their study (individuals were smaller than 100 mm). On the contrary, Frouzova et al. (2005) found a higher variation on TS data (a difference greater than 20 dB). They studied the TS of trout and perch, which have one-chambered swim bladders, and the TS backscattered from cyprinids, which have two-chambered swim bladders (Frouzova et al., 2005). These differences between the maximum and minimum TS found by Frouzova et al. (2005) could be the result of pooling different species with different swim bladders, since fish TS also depends on the size and type of the swim bladder (Ona, 1990). As discussed by Frouzova et al. (2011), TS patterns of two-chambered fish are more regular than those of one-chambered fish. Thus, TS–length equations derived from studies with mixtures of several species may include higher variations of TS.

On the other hand, we did not find any statistical difference on mean TS in either of the size classes when comparing the selected pulse lengths. This effect was already studied by Kubecka (1995), who ensonified two tethered carps (*Cyprinus carpio*) for horizontal oriented dual-beam sonars at frequencies of 200 and 400 kHz. He combined six variants of pulse duration (0.1–0.8 ms) and four variants of bandwidth (1.25–10 kHz) under laboratory conditions. Although his dual-beam results were not directly compared with ours, his conclusions indicated that pulse duration did not have significant effects on the variability of TS. Boswell and Wilson (2008) studied horizontal TS for free-swimming anchovy and menhaden fish with a Biosonics DE-X digital echo sounder equipped with a 420 kHz split beam transducer. Likewise, they did not find differences in average TS or TS distributions as a function of pulse duration. The effect of pulse duration on in situ acoustical estimates was specifically studied by Godlewska et al. (2011). Measurements were taken using a Simrad EK 60 split-beam echo sounder at a 70 kHz frequency operating vertically at four different pulse lengths (0.128, 0.256, 0.512 and 1.024 ms). In line with our results, they concluded that TS distribution was not affected by pulse duration. Hateley et al. (2013) compared six different systems in terms of frequency and manufacturers. The information obtained from sonar devices at a frequency of 200 kHz (Biosonics DTX operating at 0.4 ms of pulse length; HTI 241 at 0.2 ms and Simrad EK60 at 0.256 ms) shows that their results did not present any difference regarding Sv, even when systems differed in many parameters, including the pulse length. In conclusion, differences in TS distribution were irrelevant when using short pulse lengths and they had no effect on our acoustic results. Thus, developing specific TS–length relationships for pulse durations lower than 0.5 ms is not necessary.

To sum up, this study provides us with the horizontal conversion equations for barbel in the three main orientations. Furthermore, it is complemented by the study of some parameters that may modify the data acquisition process or the analysis of the hydroacoustic samplings. This type of study allows us to better understand the behaviour of underwater sound when used on living organisms and it improves the interpretation of the results. Therefore, we would like to encourage the scientific community to develop more studies of this kind in order to improve hydroacoustic techniques.

Acknowledgements

This study is a result of the project 082RN0801.1 funded by the Government of Spain and given to the University of Seville and Ecohydros S.L. We are grateful to Helge Balk for including the new method to calculate the track aspect in Sonar5. We would also like to thank Agustín Monteoliva, Jan Kubecka and Jarka Frouzova for their important suggestions during the course of this research. Great thanks also go to Frank Knudsen and Victor Espinosa for their theoretical assistance. We would like to thank the anonymous reviewer for improving this work with their valuable comments. We acknowledge Cesar Fallola's generosity for supplying us with the large fish. We also thank Cristina Ocaña for her careful proofreading of the English text. Thanks to all those who have contributed to this research in any way.

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CHAPTER 2:

Horizontal target strength of *Cyprinus carpio* using 200 kHz and 430 kHz split-beam systems.

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Submitted article in Fisheries Research

HORIZONTAL TARGET STRENGTH OF *CYPRINUS CARPIO* USING 200 KHZ AND 430 KHZ SPLIT-BEAM SYSTEMS

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KEYWORDS: *Cyprinus carpio*, horizontal hydroacoustics, target strength, dual-split systems, frequency.

ABSTRACT

Horizontal hydroacoustics is a useful tool to study fish in shallow waters and complete the density and biomass estimates calculated from vertical hydroacoustic samplings. In order to properly interpret the information obtained from acoustic studies, it is necessary to establish equations that relate the fish's backscattered sound or target strength (TS) to its biological parameters such as length or weight. Particularly in horizontal applications, information regarding orientation must be included in these equations since TS varies depending on swimming angles. For freshwater species, these relationships are scarce and those that are already published differ in terms of acquisition systems (single, dual or split-beam; frequencies; manufacturers), acquisition methodology (recording settings, immobilised or free-swimming fish, etc.) or species and sizes. In the density and biomass estimation process, the applied equation has an influence on the results. It is therefore necessary to develop new relationships for the species and devices most commonly used in hydroacoustic studies. In this study, conversion equations for the species *Cyprinus carpio* have been developed and compared using two different split-beam systems operating at different frequencies (200 and 430 kHz). Moreover, these equations have been compared with equations developed for dual-beam systems. The results show that the differences are greater when comparing split and dual-beam systems than when comparing frequencies. These comparisons help us establish criteria to determine the best equation to analyse our results in hydroacoustic surveys. On the other hand, studies comparing systems and frequencies are important to perform intercalibration exercises as well as to establish the foundations for the standardisation of fish samplings using hydroacoustics. These comparisons allow for a more appropriate interpretation of the information obtained from acoustic samplings and are essential for the future use and application of hydroacoustic methods.

INTRODUCTION

In previous studies, horizontal hydroacoustics has proven to be a significant tool to complete the density and biomass estimates calculated from vertical hydroacoustic samplings (Kubecka and Wittingerova, 1998; Knudsen and Saegrov, 2002). Horizontal applications are usually employed to study shallow systems, such as river systems, or superficial layers in large deep water systems since a large number of fish use these superficial habitats as a refuge, a feeding area, etc. (Encina *et al.*, 2006; Kubecka *et al.*, 2012).

In order to properly interpret the information obtained from acoustic studies, it is necessary to apply relationships between the fish's backscattered sound or target strength (TS, dB re 1 m^2), commonly used in its logarithmic form, and its biological parameters, such as length or weight. Information regarding orientation must be included in these equations, particularly in horizontal applications, given that TS varies depending on swimming angles (Hazen and Horne, 2003; Simmonds and MacLennan, 2005; Pedersen *et al.*, 2009; Jech, 2011; Rodríguez-Sánchez *et al.*, 2015b). These relationships are usually generated in previous studies where the species and the biological parameters of the individuals are known (Kubecka and Duncan, 1998; Burwen and Fleischman, 1998; Lilja *et al.*, 2000; Frouzova and Kubecka, 2004; Frouzova *et al.*, 2005; Boswell and Wilson, 2008; Rodríguez-Sánchez *et al.*, 2015a).

Horizontal TS-length relationships for freshwater species are scarce and those that are already published differ in terms of acquisition systems (single, dual or split-beam; different frequencies; different manufacturers), acquisition methodology (recording settings, tethered/anesthetised or free-swimming fish, etc.) and species and sizes. Some studies emphasize the need to apply specific equations in order to obtain reliable estimations of size distributions and biomass for hydroacoustic surveys (Godlewska, 2004; Boswell *et al.*, 2008). Therefore, it is necessary to develop new relationships between the species and devices most commonly used in hydroacoustic studies.

Furthermore, there is an increasing need to establish the foundations for the normalisation of fish samplings, partly due to the current Water Framework Directive (WFD) (2000/60/EC). The application of different frequencies could not only affect TS relationships, but echoes from a fish could also be perceived differently at each frequency. Comparisons between systems could help to understand the response obtained at each frequency. Moreover, they could be useful in establishing agreements in hydroacoustics practice. Therefore, studies of this type are essential for the future use and application of hydroacoustic methods.

This study focuses on the common carp (*Cyprinus carpio*) because of its importance in fish assemblages in the European freshwater systems. The common carp is native to Asia and it can be found everywhere in the world, except for the Middle East and the poles (Kottelat and Freyhof, 2007). This species significantly contributes to the total biomass of freshwater ecosystems due to its large size and abundance. Therefore, its acoustic study may be useful for biomass estimations. Kubecka and Duncan (1998) have already developed some relationships for carp. However, these equations were developed for two dual-beam echosounders using immobilized fish. The use of these equations with split-beam systems may produce bias in size and biomass estimations. Therefore, it would be convenient to develop new equations for the new split-beam systems, or at least to study the differences between systems.

In this study, we have contributed to the development of horizontal hydroacoustics by establishing new relationships for carp using two split-beam systems operating at different frequencies (200 and 430 kHz). These results have been compared with other horizontal equations developed for common carp using dual-beam systems. In order to determine which of the studied frequencies renders a better performance for fish detection using horizontal hydroacoustics, we have also compared the same tracks ensonified at different frequencies (200 and 430 kHz).

MATERIAL AND METHODS

The study was performed in the aquarium at the Aquatic Ecology Station of Seville, in a cylindrical pool 4 m in depth and 10 m in diameter. The transducers were placed side by side on one end of the pool so that the beam was parallel to the water surface (horizontal orientation) and aimed at the cage containing the fish. Six different size classes of common carp (*Cyprinus carpio*) were used, all of which came from the fish farm “Vegas del Guadiana” in Badajoz. They were transported under stunned conditions (Garcia-Gómez *et al.*, 2002) and kept in quarantine aquariums until their ensonification. Before the recordings, the standard length (SL, mm) and weight (W, g) of the fish were measured. Table 1 lists features for each of the studied size classes. Fish were placed individually in the cage and remained there from 48 to 96 hours to ensure that tracks from all possible orientations were obtained.

The experimental cage was a cubic frame made of PVC pipe covered by a mesh (5mm knot to knot), with a side length of 1.5 m. The cage was placed in front of the transducers at a distance of 6 m. The sound produced by the cage was studied before performing the measurements. To that end, the copper calibration sphere (13.7 mm; TS=-45 dB) was recorded at different positions. It was placed in front of the cage and in the middle of the cage. These recordings were analysed and the mean deviation in the expected TS was lower than 0.50 dB. The sound coming from the cage was insignificant (TS <-70 dB) compared to the sound of the ensonified fish and it offered excellent conditions for measuring the TS of individual fish.

Table 1
Common carp size class and recording duration.

Size class	mm)	W (g)	N	Recording duration at 200 kHz (hours)	Recording duration at 430 kHz (hours)
1	90.7	15.5	3	48	48
2	70.3	74.1	3	48	-
3	33.1	181.0	3	48	48
4	65.0	585.8	3	48	-
5	55.0	1380.0	3	48	48
6	20.0	2400.0	1	96	-

SL, mean standard length; W, mean weight; N, number of individuals

Acoustic measurements were obtained using two different systems aimed horizontally: Simrad EK60 200 kHz equipment with a 7° circular split-beam transducer and BioSonics DT6000 430 kHz system with a 4°x 8° elliptical split-beam transducer. Both transducers were mounted on a stainless steel bar fixed to the walls of the aquarium at a water depth of 2.5 m. They were placed side by side at a distance of 20 mm (measured from the centre of one transducer to the centre of the other), which was large enough to avoid interferences between systems and close enough to ensonify the same tracks (Fig. 1). These hydroacoustic systems differed in terms of sound frequency, acquisition software and beam width and shape. Therefore, both systems were set to acquire data using similar parameters in order to minimise those differences as much as possible. Transceiver settings are listed in Table 2.

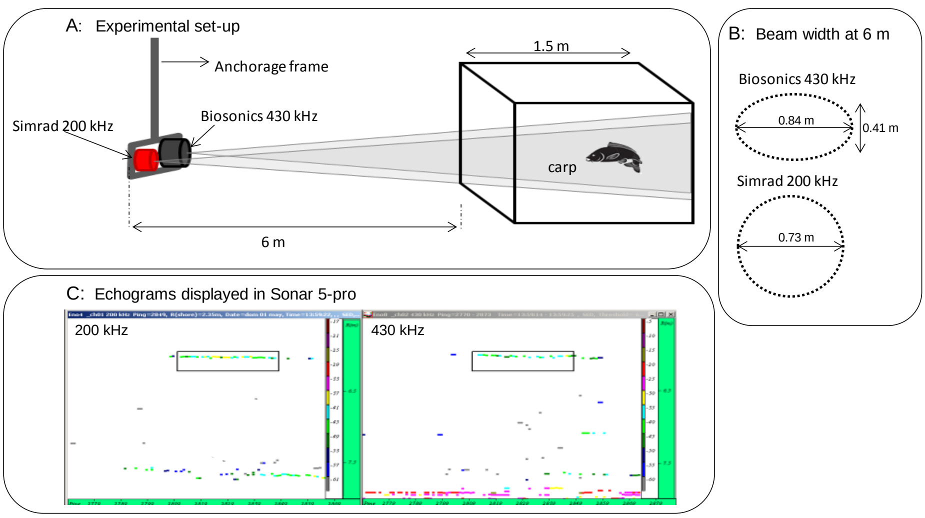

Fig.1: Schematic view of the experimental design (a). Beam width for both systems at a distance of 6 metres from the transducers (b). Example of a carp ensonified with both systems. Picture captured from the single echo detection (SED) echogram (c).

Table 2

Settings of the echosounders employed for horizontal target strength (TS) measurements.

Parameters	imrad device	Biosonics device
Type of transducer	ES200-7C	DT6000
Transducer shape	circular	elliptical
Transmission frequency (kHz)	200	430
Transmitting power (W)	150	150
Pulse length (ms)	0.128	0.100
Ping rate (ping·s^{-1})	10	10
Minimum threshold (dB)	-70	-70

Before the measurements, *in situ* calibrations were performed following the standard protocol recommended by Foote *et al.* (1987) and the equipment manuals (Simrad, 2004; BioSonics, 2004) and using the specific calibration sphere for each frequency: a 13.7 mm copper sphere for the Simrad system and a 17 mm tungsten carbide sphere for the BioSonics system.

The data recorded from free-swimming fish were stored on a PC and later processed with Sonar5 Pro v.6.0.1. analysis software (Balk and Lindem, 2011). Echograms were converted with a time varied gain (TVG) of 40 log. The echosounder's single echo parameters were set so that we obtained as many echoes as possible from the recorded fish. The minimum threshold was set to -60 dB; minimum and maximum echo lengths were 0.80 and 1.6 (rel. to pulse length). The maximum gain compensation was -3 dB (one-way) and the maximum phase deviation was 6. TS corrected for angular location in the beam (TS, dB re 1 m^2) were used for the analysis.

Tracks were manually selected and only linear swimming tracks with at least five echoes were stored. To calculate the aspects of the fish tracks, we used the method developed in Rodríguez-Sánchez *et al.* (2015a). This method used XZ positions from all echoes contained in a track to calculate the regression line on the XZ plane. The angle of the fish's swimming path with respect to the XY plane of the transducer was subsequently calculated. Once all tracks from the recordings were selected, they were classified into three main orientations depending on their swimming angle: head and tail orientation (H&T) (with angles between 0-20° and 160-180°), oblique orientation (with angles between 20-70° and 120-160°) and lateral orientation (70-120°). The mean TS of each track was calculated as the mean value of all echoes in a fish track in the linear domain.

Statistical analysis

The acoustic data collected were statistically analysed by SPSS 20.0. (IBM Corp., 2011). For both frequencies, the measured TS values were regressed against the logarithm of the length using the following relationship:

$Y = a \cdot LogX + b$, where Y is the target strength (TS) in dB, X is the length of the fish and a and b are regression constants.

An analysis of variance (ANOVA) was conducted for each studied frequency in order to test the TS differences in relation to the size of the fish. In order to study the variation of TS depending on the aspect angle, a one-way analysis of covariance (ANCOVA, α=0.05) was conducted with the standard length as the covariate. Differences between TS measurements recorded at the two different frequencies were tested using an analysis of covariance (ANCOVA, α=0.05) with the standard length as the covariate.

In order to analyse the differences between the positions of the tracks calculated at each applied frequency, a Sonar5-Pro multi-frequency analysis tool was used since it allows selecting the same track at both frequencies. A total of 100 tracks were selected for analysis**.** Based on the XZ positions of the echoes of the tracks (X=along-ship, Z=range), the regression lines that represent the fish paths were calculated. The goodness of fit of the position regressions (R^2) was compared for each pair of tracks (with the term "pair of tracks" referring to the same track at 200 kHz and at 430 kHz). The differences found between the position estimates concerning the range (position Z in the XYZ space) were not taken into consideration in this study because they were the result of the differences in the shape and size of the transducers. Given that the transducer used at 200 kHz is 10 mm shorter than that used at 430 kHz, the same fish was closer to the 430 kHz transducer than to the one at 200 kHz.

RESULTS

The mean TS (dB) varied significantly at both frequencies in both species, depending on the size class (ANOVA $F_{5.1256}$=424.075; p<0.001 for 200 kHz and $F_{2.692}$=178.051; p<0.001 for 430 kHz). The results show that TS is directly related to fish length. Specifically, TS tends to rise as the fish length increases. The fish length was therefore used as a covariate for the subsequent analysis. On the other hand, TS also changed at both frequencies as a consequence of the variations produced in the aspect angle or swimming orientation (ANCOVA; $F_{17.1251}$=89.566 p<0.001 for 200 kHz and $F_{17.689}$=13.953; p<0.001 for 430 kHz).

Figure 2 represents an example of these changes in TS at 200 kHz in two of the studied size classes. It was observed that size class 2 (with standard fish lengths ranging from 100 to 200 mm) presents lower TS values than those found in size class 4 (with individuals ranging from 300 to 400 mm) in every orientation. On the other hand, the TS of fish with head and tail orientations (H&T) present the lowest values regardless of the size of the fish. These values increase as fish swim more laterally. Within each size class, the differences found between the maximum and minimum TS values due to swimming orientation were lower than 11 dB at both frequencies. The maximum TS values were obtained in lateral orientations (with angles ranging from 70 to 120°).

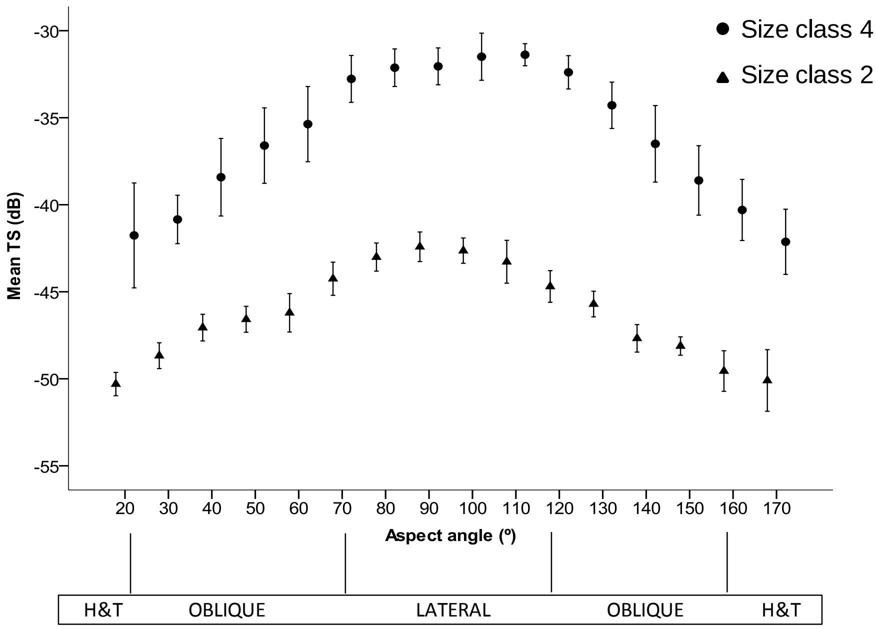

Fig 2. Distribution of mean TS and confidence interval (95%) obtained at 200 kHz for size classes 2 and 4 along the different swimming orientations.

The equations of horizontal conversion between TS and length for carp have been calculated separated by frequency. Significant regressions were obtained for the three main orientations at both frequencies (ANOVA; p<0.001 for head and tail, oblique and lateral orientations) (Table 3). All equations showed a high correlation (R^2) between the dependent variable TS and the related independent variable standard length.

The mean TS obtained at 200 kHz and 430 kHz were significantly different (ANCOVA $F_{1.1946}$=16.223; p<0.001). TS values at 200 kHz were higher than those obtained at 430 kHz, although these differences were not highly pronounced (they were lower than 4dB) (Table 4). The same occurred when the mean TS responses obtained for the main orientations were separately compared by frequency (Table 5). The TS response registered at 200 kHz was stronger than that obtained at 430 kHz for all orientations and the differences in mean TS were not pronounced (Table 4).

Table 3
Horizontal linear regressions of target strength (TS, dB) vs. standard length (SL, mm) for carp ensonified with two split-beam systems operating at different frequencies.

Results of the linear regressions calculated for the three main horizontal orientations at 200 kHz							
Orientation	X	*a*	*b*	F	*df**	*P*	R^2
H&T	Log SL	22.54	-96.69	748.75	1.193	<0.001	0.796
Oblique	Log SL	23.60	-95.39	2680.18	1.690	<0.001	0.795
Lateral	Log SL	24.63	-93.97	3342.82	1.378	<0.001	0.899
Results of the linear regressions calculated for the three main horizontal orientations at 430 kHz							
H&T	Log SL	21.69	-96.16	13.76	1.60	<0.001	0.723
Oblique	Log SL	23.16	-96.58	297.94	1.211	<0.001	0.587
Lateral	Log SL	23.61	-93.78	143.58	1.120	<0.001	0.547

**df*, degrees of freedom

Table 4
TS values for the different orientations separated by frequency. Number of tracks analysed (N). Minimum, maximum and mean TS in dB and standard deviation (Std. Dev.)

Frequency	Orientation	N	Minimum	Maximum	Mean	Std. Dev.
	H&T	194	-52.16	-32.52	-42.73	5.43
200 kHz	Oblique	691	-55.78	-27.69	-41.93	6.55
	Lateral	379	-50.68	-26.04	-37.85	6.28
	All data	**1256**	**-55.78**	**-26.04**	**-40.75**	**6.59**
	H&T	140	-56.00	-37.62	-47.21	4.46
430 kHz	Oblique	342	-55.54	-28.90	-45.46	5.03
	Lateral	208	-52.89	-30.76	-41.06	4.46
	All data	**692**	**-56.00**	**-28.90**	**-44.55**	**5.53**

Table 5
ANCOVA on mean TS separated by orientation and compared by frequency.

Orientation	*df**	F	*P*
H&T	1.253	76.28	<0.001
Oblique	1.902	52.00	<0.001
Lateral	1.498	22.28	<0.001

* *df*, degrees of freedom

Searching and storing tracks at 430 kHz was much more difficult than at 200 kHz. When the echograms were studied, it was noted that tracks recorded at 200 kHz were better defined than those recorded at 430 kHz. Tracks at 200 kHz showed a smaller number of missing echoes and a lower dispersion in the XZ positions.

In the comparative study of positioning between frequencies, two different situations were found. These situations are summarized in Fig. 3. Fig. 3(a) shows the track of a fish ensonified at 200 and 430 kHz moving from one side of the beam to the other. In this case, the direction of travel is well characterized at both frequencies, although the regression resulting from the XZ positions showed a better fit (R^2) at 200 kHz than at 430 kHz. In Fig. 3(b), the XZ positions of the track are correctly determined at 200 kHz (R^2=0.940; p<0.01), while the positions at 430 kHz are not well characterized and the swimming trajectory of the fish is unknown (R^2=0.289; p>0.01). Moreover, in this case, the regression of the XZ positions calculated at 430 kHz was not significant.

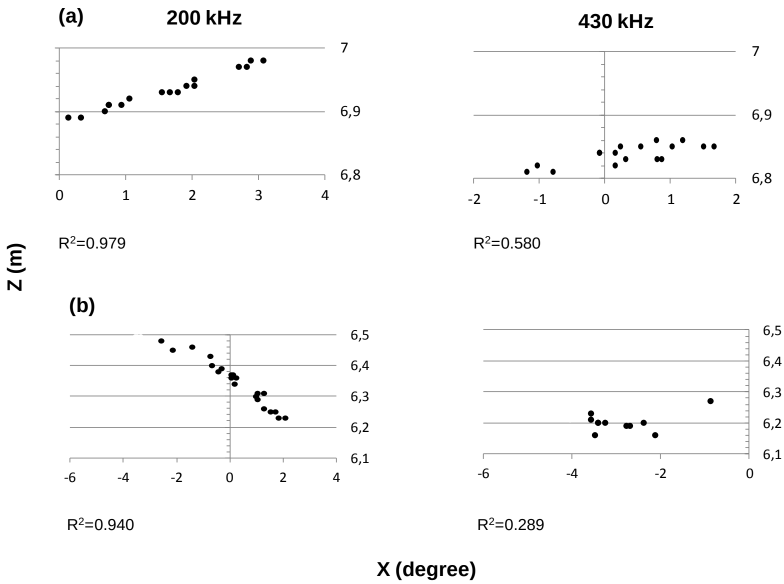

Fig. 3 Graphical representation of the XZ positions of the same track simultaneously ensonified by both studied systems (200 and 430 kHz). The positions are expressed as a time series of X and Z distances from the centre of the split-beam systems.

In 80% of the analysed pairs of tracks, the fit of the regressions of XZ positions found at 200 kHz was better than at 430 KHz, regardless of their statistical significance. When the tracks with significant regressions were selected (70% of cases at 200 kHz and 45% of cases at 430 kHz), only 12% of the tracks recorded at 200 kHz registered a fit lower than 0.500, whereas at 430 kHz 41% of the cases registered a fit lower than 0.500.

DISCUSSION

The choice of the appropriate equation for the conversion between TS and fish length is fundamental for hydroacoustics. The application of equations developed for species other than those surveyed may produce bias in the density and biomass estimates (Boswell *et al.*, 2008). Moreover, when applying horizontal hydroacoustics, the results show that variations in the fish's swimming angles result in changes in the backscattered energy and, as a consequence, the TS backscattered from a lateral aspect is greater than that backscattered from a head or tail position. Therefore, in horizontal applications, the equations for TS conversion must be specifically developed for each of the main aspects: head and tail, oblique and lateral

(Ona, 1990; Simmonds and MacLennan, 2005; Jech, 2011).

The TS-length relationships developed in this study for *Cyprinus carpio* using split-beam systems operating at different frequencies (200 and 430 kHz) will help to improve the results of hydroacoustic studies performed in systems where the carp population constitutes an important component of the fish community. Furthermore, these relationships will increase the number of equations available for the conversion of hydroacoustic data obtained using horizontal hydroacoustics.

As occurs with species, the application of an equation developed for systems or frequencies other than those used in the survey may produce differences in density and biomass estimates. In order to study this effect, we have compared the lateral TS-length equations for common carp developed by Kubecka and Duncan (1998) using dual-beam systems with those obtained in this experiment (Fig. 4). It was observed that, for the same TS value, the length values obtained using the dual-beam system were lower than those obtained using split-beam systems at the same frequency. These differences imply that, for instance, a TS value of -31 dB corresponds to a fish size of approximately 100 mm using the equation for dual-beam systems, while the split-beam system relationship gives a length value of around 400 mm, which entails a weight difference of approximately 1,500 g.

Given that the comparison was performed between systems that work differently (dual vs. split-beam systems), certain differences were expected. However, they were not expected to be so large. In agreement with Godlewska (2004), we believe that these variations could be more pronounced than expected due to the methodology used for data acquisition. Kubecka and Duncan (1998) ensonified immobilised fish placed on the acoustic axis. They obtained the TS measurements from the most reflective area of the fish (i.e. from the area covered by the swim bladder oriented to the central part of the transducer), which could result in TS values greater than those found in our experiment where fish swam freely. In our case, the selected tracks could include echoes coming from different parts of the fish's body, which, in summary, could increase the TS variation and decrease the mean TS value.

Likewise, the fish's backscattered energy is received in a different way at different frequencies and, thus, differences in conversion equations can be expected. One can expect higher TS at higher frequencies (Dahl and Mathisen, 1983), but our results showed that the mean TS was greater at 200 kHz than at 430 kHz in every studied size, which is consistent with Kubecka and Duncan's (1998) results. Apart from the specific characteristics of each system, the disparity between frequencies could be explained by the differences in the directivity property. Directivity may produce losses of energy at high frequencies and, as a consequence, lower TS values may be recorded. Nevertheless, the differences found between frequencies were lower than those found when comparing dual with split-beam systems.

In line with these results, we would like to highlight the problems associated with the 430 kHz frequency during the track collection process. In most cases, the path of fish was reproduced more accurately at 200 kHz and their tracks had a higher number of echoes. The results also showed that the fit of the regressions obtained for track positions were better at 200 kHz. As mentioned above, the 430 kHz system is more directive than the 200 kHz system and it might be possible that, at the studied distances (6-7.5 m), the main beam could not record all of the backscattered energy from the fish and, consequently, the system lost echoes from the fish. In other cases, the 430 kHz system was unable to properly locate the track in the beam. This leads us to believe that systems with higher frequencies could be less effective in horizontal hydroacoustics studies where the position of the track is fundamental for size and biomass estimates. Based on our results, applying hydroacoustic systems with a frequency of 200 kHz is recommended when conducting horizontal hydroacoustic measurements at close ranges.

Our results show that, in horizontal hydroacoustics, the target strength of a fish is not only related to the length of the fish, but is also strongly influenced by the applied system, the frequency and the swimming aspect with respect to the sound source. In any case, comparisons such as the one presented here are fundamental. On the one hand, they contribute to the general knowledge of hydroacoustics and provide us with criteria to determine which system is the most appropriate to study a particular ecosystem. On the other hand, they may be helpful for the intercalibration process and for establishing the foundations for the normalisation of fish techniques, which are essential in light of European regulations such as the Water Framework Directive (WFD).

ACKNOWLEDGEMENTS

This study is the result of project 082/RN08/01.1, which has been funded by the Government of Spain and commissioned to the University of Seville and Ecohydros S.L. We would like to thank Jan Kubecka and Jarka Frouzova for their important suggestions regarding the setup of the experiment. Great thanks also go to Frank Knudsen and Helge Balk for their theoretical assistance. We acknowledge Cesar Fallola's generosity for supplying us with the fish. Special thanks go to all those who have contributed to this research in any way.

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CHAPTER III

Do close range measurements affect the target strength (TS) of fish in horizontal beaming hydroacoustics?

Rodríguez-Sánchez, V., Encina-Encina, L., Rodríguez-Ruiz, A., Sánchez-Carmona, R. (2015). Article in press: Fisheries Research (2015), http://dx.doi.org/10.1016/j.fishres.2015.03.020

G Model
FISH-4091; No. of Pages 7

ARTICLE IN PRESS

Fisheries Research xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Do close range measurements affect the target strength (TS) of fish in horizontal beaming hydroacoustics?

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ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form 24 March 2015
Accepted 26 March 2015
Available online xxx

Keywords:
Horizontal beaming hydroacoustics
Near-field
Target strength
Fish

ABSTRACT

Detailed fish target strength (TS) studies allow us to relate their physical and biological variables to their sound behaviour and they potentially improve the accuracy of acoustic assessments. One of the limitations of the transducers that are currently used is their difficulty to precisely estimate TS at close range, either because the acoustic beam may not include the whole fish or because of the near-field effect. When measuring the sound produced by a target (fish), it is recommended that the so-called near-field area, which is generated immediately in front of the target, should be avoided. In horizontal hydroacoustics, where information is obtained within the first 15–20 m from the transducer, avoiding this near-field area may render the majority of the sampled volume useless. This experiment was developed in order to study the horizontal behaviour of the TS with regard to distance. We have studied three different sizes of free-swimming large carps and barbels (≈300, 400 and 500 mm) and we have recorded fish traces at three distances from the transducer, both fulfilling and not fulfilling the requirements of the near-field (6, 9 and 12 m). The results showed no differences in the mean TS obtained for fish at different distances. Factors such as orientation or size showed a greater influence on TS changes. These results are very encouraging and they support the use of hydroacoustics in studies performed at close range, such as studies of fish migration in rivers or biomass estimations in aquaculture.

1. Introduction

Hydroacoustics is a technique used as a tool for fish research both in freshwater (Yule, 2000; Wanzenbock et al., 2003; Godlewska et al., 2012, Dennerline et al., 2012) and marine systems (Fabi and Sala, 2002; Neilson et al., 2003; Axenrot et al., 2004; Greenstreet et al., 2010). In order to accurately assess fish density and biomass, it is essential to establish the relationship between the intensity of the sound backscattering from fish, which is known as target strength (TS dB re 1 m^2), and the fish variables such as length or weight (Simmonds and MacLennan, 2005).

The use of hydroacoustics as a tool to study water systems is increasing since it has numerous advantages compared with other traditional methods. One of the most relevant advantages is that it is a non-invasive application that covers large areas with high spatial resolution and low manpower requirements (Simmonds and MacLennan, 2005; Godlewska and Jelonek, 2006). In spite of the advantages of hydroacoustic methods, there are some disadvantages that may complicate the acquisition of accurate estimates of fish TS, especially in horizontal applications (with the transducer positioned parallel to the water surface).

Horizontal applications involve greater complexity and uncertainty than vertical applications since changes in the fish swimming orientations lead to significant TS changes. It is known that large variations have been encountered when fish swim from a lateral position to a head or tail position (Kubecka, 1994; Lilja et al., 2000, 2004; Frouzova et al., 2005; Boswell and Wilson, 2008; Pedersen et al., 2009; Rodríguez-Sánchez et al., 2015). Therefore, when horizontal applications are used, the study of these variations and their inclusion in TS-length relationships are fundamental.

Horizontal hydroacoustics is usually used to complement or complete vertical density and biomass estimates when, for example, fish are aggregated close to the surface or in shallow areas (Kubecka and Wittingerova, 1998; Yule, 2000; Knudsen and Saegrov, 2002). In these shallow systems, TS measurements may present deviations due to boundary effects (Mulligan, 2000) and a low signal-to-noise ratio (SNR) (Kieser et al., 2000). Boundary effects are produced by the direct reverberation of the sound wave that comes from the bottom and/or the surface of the water,

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http://dx.doi.org/10.1016/j.fishres.2015.03.020
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which generates a noisy background. This affects the signal-to-noise ratio (SNR), which is the magnitude of the difference between the echoes coming from fish and the "unwanted" echoes coming from other non-fish sources within the water column (Simmonds and MacLennan, 2005). Therefore, when analyzing these systems, it is also important to take both effects into consideration.

On the other hand, one of the limitations of the transducers that are currently used is that the precise estimation of TS at close range is difficult, either because the acoustic beam may not include the whole fish or because of the near-field effect. The near-field is an unstable sound area that is generated close to the sound source in any object that emits a sound wave (Medwin and Clay, 1998). Therefore, this area is usually avoided in order to prevent bias in biomass estimations (Dawson et al., 2000; Mulligan, 2000; Simmonds and MacLennan, 2005).

Besides the near-field of the acoustic transducers, the ensonified fish also have a near-field that is directly related to their length (i.e., large fish have large near-fields) (Medwin and Clay, 1998). The combined near-fields of the transducer and the fish can be a substantial proportion of the distance between the transducer and the fish and they could render hydroacoustics useless because of the reduction of the effective ensonified volume, especially in systems where large fish are an important component of the fish community. In summary, horizontal sampling is limited by the noise and the volume available for analysis is considerably reduced when the usable volume is limited by the SNR and the near-field area is removed.

Studies dealing with this issue are scarce and there is little information about how the aforementioned theoretical limitations affect real data and whether the bias of the combined near-fields of the transducer and the fish leads to serious errors in the collected data. In this study, two species were used to test this effect: *Luciobarbus sclateri* and *Cyprinus carpio*. These species were selected because of their importance in fish assemblages in European freshwater systems (Encina et al., 2006). Due to their large size and abundance, both species significantly contribute to the total biomass of these systems and, therefore, their acoustic study may be useful for biomass estimations.

The aim of this study was (i) to evaluate TS changes of large barbels and carps in relation to their swimming angles, species and distances from the transducer and (ii) to determine which of the studied distances produce reliable measurements of TS for large fish.

2. Material and methods

2.1. Fish collection and experimental setup

The study was conducted with two fish species: Andalusian barbel (*Luciobarbus sclateri*) and common carp (*Cyprinus carpio*). Specimens of these two species were collected from anglers in the inner harbour of the Guadalquivir River. After collection, fish were anaesthetised with clove oil to prevent injury during transportation to the experiment site (García-Gómez et al., 2002). Before the recordings, the standard length (SL) and weight (W) of the fish were measured in millimetres and grams, respectively.

Three individuals from each species were selected for the experiment. The standard lengths of the ensonified fish were ≈300, 400 and 500 mm (Table 1). The fish were placed individually in the experimental cage so that overlapping echoes from multiple targets were not falsely accepted as valid echoes from the same track. They remained in the cage for 24–96 h in order to obtain enough data for the statistical analysis.

The experimental cage was a cube with a side length of 1.5 m. It was made from PVC pipe with holes in order to let the water pass inside, thereby avoiding undesirable air bubbles (Hartman and Nagy, 2005). The cage was covered by a mesh (5 mm, knot to knot). A calibration test was performed in order to ensure that there were no significant echo returns coming from the cage. The test involved performing a horizontal calibration for all the studied distances and collecting recordings of the calibration sphere at three different positions with respect to the cage: in front of the cage, in the middle of the cage and behind the cage. Phase files of these recordings were analysed and the mean phase deviation (−6 dB level) was lower than 0.45 for both Athwart-ship and Along-ship positions in every case.

The frame was located in front of the transducer at the selected distances: 6, 9 and 12 m. The first distance was set at 6 m in order to allow the diameter of the acoustic beam cross section for a complete ensonification of the largest fish. The cage was placed in the old lock of the inner harbour of the Guadalquivir River. This location provided us with an area protected from the wind and tidal current, which is ideal for performing TS experiments (Gangl and Whaley, 2004).

TS data were recorded with a Simrad EK60 scientific echosounder (Simrad Kongsberg Maritime AS, Horten, Norway) equipped with a 200-kHz circular split-beam transducer (ES200-7C). The transducer was aimed horizontally, parallel to the water surface. It was fixed on a stainless steel bar anchored to the bottom by ballast at a depth of 1.5 m. In addition, the anchored bar was tied to a tree with ropes in order to maintain its position (Fig. 1). The transducer depth was selected so that the main axis could cross the centre of the cage. The acoustic unit was calibrated with a 13.7-mm copper sphere (TS = −45 dB) following the standard calibration method (Foote et al., 1987). Transceiver settings are listed in Table 2. Data were stored on a PC and later processed with the Sonar5 Pro v.6.0.1 analysis software (Balk and Lindem, 2011).

2.2. Acoustic processing and near-field calculation

In order to eliminate noise and to ensure that we obtained as many detections as possible, the single echo detector parameters for the echosounder were set to accept echoes with the settings

Table 1
Near-field distances calculated for three different sizes of barbel and carps and state of the theoretical requirements for accurate TS measurements.

	SL (mm)	SbL (mm)	D_{SL} (m)[a]	D_{SbL} (m)[a]	State of requirements					
					Relative to SL			Relative to Sb		
					6 m	9 m	12 m	6 m	9 m	12 m
Barbel	350	106.0	4.37	0.38	F	F	F	F	F	F
	420	124.0	6.04	0.52	U	F	F	F	F	F
	500	150.0	8.56	0.77	U	U	F	F	F	F
Carp	325	112.3	3.61	0.43	F	F	F	F	F	F
	400	142.0	5.47	0.69	U	F	F	F	F	F
	525	181.0	9.43	1.12	U	U	F	F	F	F

SL, standard length; SbL, swim bladder length; D_{SL} near-field calculated from standard length; D_{SbL} near-field calculated from swim bladder length; F, fulfilled requirements; U, unfulfilled requirements.
[a] The near-field of the transducer(0.65 m) needs to be added to the near-field calculated from both standard and swim bladder length in all distances.

Table 2
Settings of the echosounder during horizontal target strength (TS) measurements.

Type of transducer	ES-200-7C
Transmission frequency (kHz)	200
Transmitting power (W)	150
Pulse length (ms)	0.128
Ping rate (ping*s^{-1})	10
Minimum threshold (dB)	−70

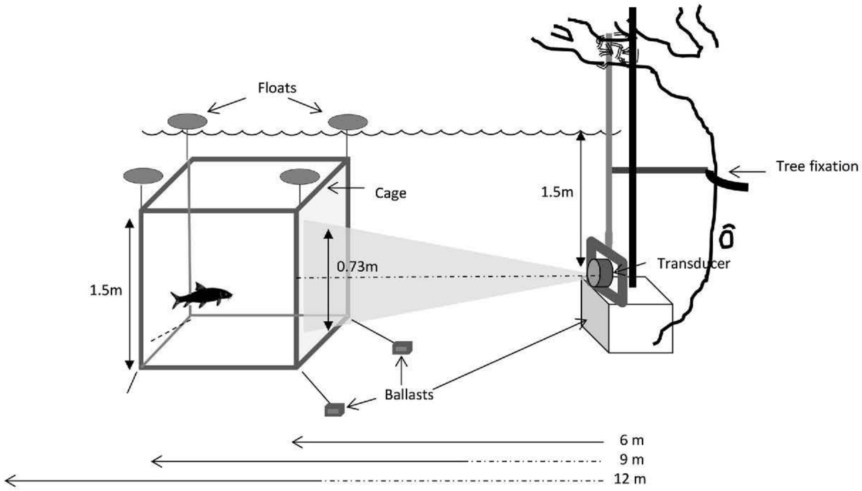

Fig. 1. Schematic view of the experimental setup. The transducer is mounted in front of the cage, which is fixed to the bottom by four ballasts and suspended by four floats.

Table 3
Single echo detector settings used in the target strength analysis.

TVG	40logR
Minimum TS (dB)	−55
Min/max echo length (rel. to pulse length)	0.8/1.6
Maximum gain compensation (one way) (dB)	−3

listed in Table 3. The analysis used TS measurements corrected depending on their angular location in the beam (i.e. off-axis compensated TS).

Tracks were selected using the manual tracking tool from the analysis menu (Balk and Lindem, 2011). In order to be accepted as valid tracks, the targets had to be separated from the previous ones by more than three pings and each track needed to contain at least five echoes (Henderson et al., 2007). Given that natural fish movements produce changes in TS, only linear fish tracks were analysed in order to filter those convex-concave oscillations and reduce bias in TS (Pedersen et al., 2009).

At the studied distances (6, 9 and 12 m), the volume occupied by the beam was included in the volume occupied by the cage, i.e. the beam did not protrude out of the cage. Thus, we stored fish tracks from the entire beam width (−3 dB one way).

The mean TS of each track was calculated as the mean value of all echoes in a fish track. Mean TS was calculated in the linear domain as follows:

$$MfeanTS = 10 \cdot \log\left(\frac{1}{N}\sum_{i=1}^{N}10^{TSi_i/10}\right) \quad (1)$$

The aspects of the fish tracks were calculated using the specific method developed by Rodríguez-Sánchez et al. (2015) and implemented in Sonar5-Pro. This method employs information from the whole track, removing the Y component of the track vector. The method uses XZ positions from all echoes to calculate the regression line in the XZ plane. Then, it calculates the angle of the ensonified fish's swimming path with respect to the XY plane of the transducer. The aspect where the fish axis was perpendicular to the acoustic axis was fixed at 90° (lateral aspect). The recorded tracks were divided into 18 categories of 10° according to their aspect angle. Afterwards, the mean TS of each category was calculated. Subsequently, three angular regions were established and renamed according to their orientation: head/tail orientation (comprising angles between 0°–20° and 160°–180°), oblique orientation (with angles between 20°–70° and 120°–160°) and lateral orientation (70°–120°).

The near-field of the applied transducer was 0.65 (Tichy et al., 2003). The fish near-field was calculated for each species' length (SL) by means of the following equation:

$$D = r^2/\lambda, \quad (2)$$

where r is the half-length of the fish (SL) and λ is the length of the sound wave (Medwin and Clay, 1998). The calculated wave length λ at 200 kHz was $7.3{*}10^{-3}$ m.

In order to recalculate the near-field distances from the area occupied by the swim bladder as well as to complement the acoustic measurements with information about the shape and volume of the swim bladder, fish were laterally and dorsally X-rayed after sound measurements. A portable Diagnostic X-ray Unit EcoRay Orange 1060 HP was used to perform the radiographs at an intensity of 4 mAs and 55 kv during 1.2 s. The lateral area of the swim bladder (A) and the lateral area of the body (B) were calculated for each fish (Fig. 2). We considered the lateral area of the fish to be only from the mouth to the root of the tail (standard length) because the fish tail scatters sound very weakly (Kubilius and Ona, 2012). The swim bladder area (A) and the body area (B) of the fish were calculated using the public domain Java image processing and analysis program IJ 1.46r (Rasband, 2014). The ratio between areas was calculated as follows: swim bladder area divided by body area and multiplied by 100.

Table 1 shows the results of the near-field calculations and it specifies whether the theoretical requirements for accurate measurements of TS are fulfilled (F) or unfulfilled (U). When the conditions are fulfilled, the sum of the near-fields of the fish and the transducer is shorter than the distance between the fish and the transducer and, therefore, data should not be affected by the near-field effects. The opposite occurs when the conditions are unfulfilled. The first size (≈300 mm) was used as a reference since

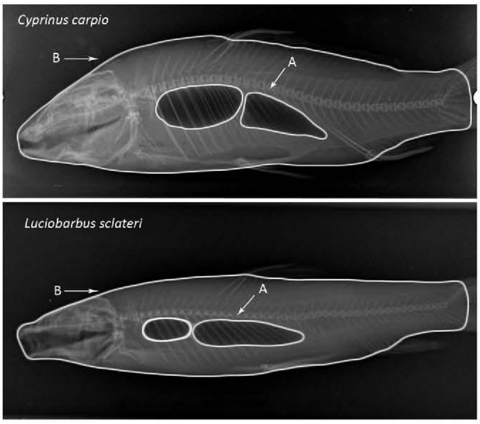

Fig. 2. Radiographs of *Cyprinus carpio* and *Luciobarbus sclateri* collected for this experiment in the inner harbour of the Guadalquivir River. The swim bladder area (A) and the body area (B) are highlighted.

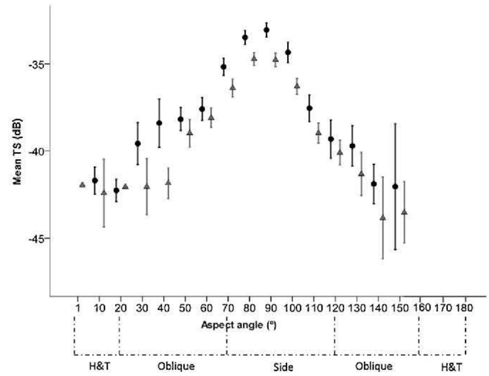

Fig. 3. Mean TS obtained for all the studied sizes classified by fish aspect angle and 95% interval confidence. The solid circles represent mean TS for carp and the grey triangles represent mean TS for barbel.

it fulfilled the theoretical distance requirements for accurate TS measurements in every case.

2.3. Statistical analysis

The lateral TS values of each species were regressed against the logarithm of the standard length using the following relationship:

$$TS = a * \log_{10} L + b \quad (4)$$

where TS is the target strength in dB, L is the standard length (mm) and a and b are regression constants.

A one-way analysis of covariance (ANCOVA) was conducted using all the selected tracks in order to test the effects of swimming orientation and species on the mean TS, with the total length as the covariate. Before the analysis, the homogeneity-of-regression (slope) assumption was tested.

A Kolmogorov–Smirnov goodness-of-fit test was used to test the normality of the lateral samples at each of the studied distances. As we expected that the near-field effect would not be constant for all distances and species, we conducted a two-way ANCOVA in order to study the influence of these two factors on the mean TS results, with the logarithm of the standard length as the covariate. Only mean TS obtained from fish lateral aspects were included in the analysis since lateral orientation exposes the largest area of swim bladder to sound. Every statistical analysis was performed by means of SPSS 20.0 (IBM SPSS, 2011).

3. Results

3.1. Effects of body length, body aspects and species on mean TS

In total, 764 tracks of barbels (397 with lateral orientation) and 844 of carps (493 with lateral orientation) were analysed. Significant linear regressions between lateral TS and standard length were obtained for both carps and barbels ($p < 0.001$; $R^2_{carp} = 0.617$ and $R^2_{barbel} = 0.452$). Therefore, the length was used as a covariate for subsequent analysis.

The homogeneity-of-regression assumption indicated that the relationship between the covariate and the dependent variable TS did not differ significantly as a function of swimming orientation ($F_{14.771} = 0.033$, $p = 0.968$ for barbel and $F_{15.746} = 1.019$, $p = 0.431$ for carps) or species ($F_{1.1515} = 0.022$, $p = 0.881$). The mean TS was significantly influenced by the aspect angle (ANCOVA; $F_{14.676} = 29.543$, $p < 0.001$ for barbel and $F_{15.715} = 22.224$, $p < 0.001$ for carp, Fig. 3) and, consequently, changes in the fish swimming direction resulted in mean TS differences. The pattern of TS changes was similar for both carps and barbels, with an increase of TS as fish moved from a head/tail swimming position to a lateral position. The difference between the maximum and the minimum mean TS was almost 10 dB (Fig. 3). In both species, the highest TS values were obtained from lateral orientations and, among these lateral orientations; the highest values were obtained for angles ranging from 80° to 100°. There were significant differences in mean TS values between species (ANCOVA: $F_{1.1516} = 71.808$, $p < 0.001$). Although the studied size range was similar between the species (Student's t test; $p = 0.895$), mean TS of carps was higher than that obtained for barbels in all aspects (Fig. 3). The average TS value for carps was −33.79 (SD = 3.39) and the average TS for barbels was −35.34 dB (SD = 3.25).

Both barbels and carps had two-chambered swim bladders. The ratio of the swim bladder and the lateral body areas ranged between 11 and 12.5% in carps and between 9 and 10% in barbels. The ratios of carps were always higher than those of barbels, with an average difference of 2%. This means that the proportion of the body occupied by the swim bladder was always larger in carps than in barbels.

3.2. TS distributions at different distances from the transducer

Lateral TS had a normal distribution and similar histograms in each of the studied species and distance combinations (Fig. 4). The results of the two-way ANCOVA used to test the influence of species and distance on the resulting TS are shown in Table 4. There is no significant interaction between the studied factors (species vs. distance) and, therefore, each of them can be independently interpreted. On the one hand, the mean TS for barbels was lower than that of carps at every studied distance ($p < 0.001$). On the other hand, there were no significant differences in the mean TS obtained at each of the studied distances ($p = 0.133$), regardless of species. In other words, TS values changed depending on the species, but they did not change with distance in any of the studied lengths. Thus, none of the sizes presented significant changes along distance.

For each specific lateral aspect angle and species, differences in TS among the studied distances were lower than 1 dB, with the exception of carps with aspects ranging from 70° to 80° (Fig. 5). All

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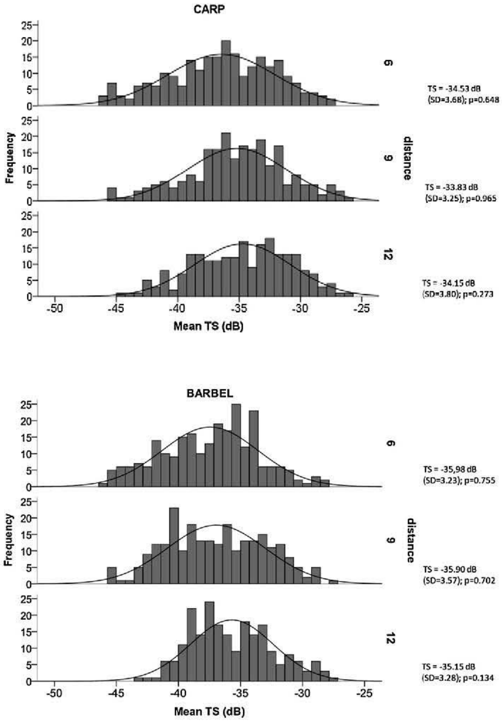

Fig. 4. Histograms of lateral TS recorded from all studied sizes of carp and barbel at different distances. Mean TS, standard deviation (SD) and significance (p) for the Kolmogorov–Smirnov goodness-of-fit test.

Table 4
Effect produced by the fish species and the distance between the fish and the transducer on the resulting TS (with the logarithm of the standard length (mm) as the covariate).

Parameter	Sum of squares	d.f.	F	P
Log SL	1122.7	1	102.05	<0.001
Species	894.4	1	81.30	<0.001
Distance	44.4	2	2.02	0.133
Species*distance	60.1	2	2.73	0.066
Error	11,100.8	1009	–	–
Corrected total	12,924.3	1015	–	–

d.f., degree of freedom.

distances gave the maximun TS values when the fish were swimming with orientations between 80° and 100°. The mean TS did not present any difference among the studied distances, regardless of the total length of the specimens.

4. Discussion

Previous studies have demonstrated the need to include horizontal beaming as a part of hydroacoustic fish stock surveys in natural freshwater systems (Kubecka and Wittingerova, 1998; Knudsen and Saegrov, 2002). In this regard, apart from establishing relationships between biological parameters and acoustic data, it is very important to understand sound behaviour when horizontal beaming is applied.

Our results prove that factors such as species, length and swimming orientation have a significant influence on the results of TS estimates. Therefore, they must be taken into account when analyzing acoustic data in horizontal samplings.

In agreement with previous studies, we confirm the importance of fish swimming orientation as a factor that affects the TS values in horizontal applications (Foote, 1980; Kubecka and Duncan, 1998; Burwen and Fleischman, 1998; Hazen and Horne, 2003; Frouzova et al., 2005; Henderson et al., 2007; Jech, 2011; Kubilius and Ona, 2012; Rodríguez-Sánchez et al., 2015). Lateral orientations gave consistently greater TS values than head and tail orientations. Therefore, information about orientation must be included in horizontal TS studies in order to obtain true fish biomass estimations. On the other hand, the accuracy of the results of acoustic samplings performed with horizontal orientations depends on the development of specific TS-length relationships.

The near-field area had already been identified as an issue that endangers the effectiveness and validity of horizontal hydroacoustics since fish are recorded at ranges where the theoretical requirements are unfulfilled (Dawson et al., 2000; Knudsen et al., 2004; Kubilius and Ona, 2012). Therefore, it was necessary to test the effectiveness of horizontal hydroacoustics at close range. It was not reasonable that a technique used in shallow areas and narrow channels or rivers had to avoid a near-field distance longer than 10 m with fish larger than 500 mm in order to record reliable data.

According to our results, the horizontal TS of large fish ensonified in lateral positions did not seem to depend on distance. After measuring the TS of different targets at different ranges, Dawson et al. (2000) and Knudsen et al. (2004) concluded that the standard near-field formulas overestimate the proportion of space occupied by the near-field effect. According to Knudsen et al. (2004) and the results obtained in this experiment, this voiding effect could be explained by swim bladder function, because it is responsible for most of the backscattered fish energy (Foote, 1980; Hazen and Horne, 2003). In our study, when the near-field is calculated based on the swim bladder, the theoretical requirements are fulfilled in every studied size and distance and the TS of specimens does not vary. This supports the theory that near-field calculation is overestimated and that it could be calculated based on the swim bladder. Moreover, if the near-field is calculated by taking swim bladder size into account rather than total body size, the volume available for the analysis of waterbodies increases, as does the applicability of horizontal hydroacoustics in acoustic samplings.

On the other hand, it could be expected that the TS of a big fish partially ensonified at close range be different from the TS obtained by the same fish from a greater distance, where the beam width would allow for its entire ensonification. However, our study shows that the TS of fish ensonified at close range is the same as that obtained from a greater distance. Therefore, at close ranges, if the swim bladder is included in the acoustic beam, the resulting TS could represent the whole fish, even when the size of the ensonified fish exceeds the diameter of the beam.

The fact that mean TS is lower in barbels than in carps is in accordance with the calculations of the swim bladder ratio since a barbel's swim bladder occupies a smaller relative volume than that of a carp. According to Frouzova et al. (2005) and Boswell and Wilson (2008), those differences in mean TS between species were most likely a result of the specific morphology of the species. However, those differences were not significant enough to render species discrimination feasible because, as the radiographs showed, morphological differences between species were not pronounced.

Our results highlight the usefulness and accuracy of density and biomass estimates obtained by means of horizontal hydroacoustics. They clarify previous speculations and represent a step forward in the understanding of sound behaviour for the detection of fish in shallow systems.

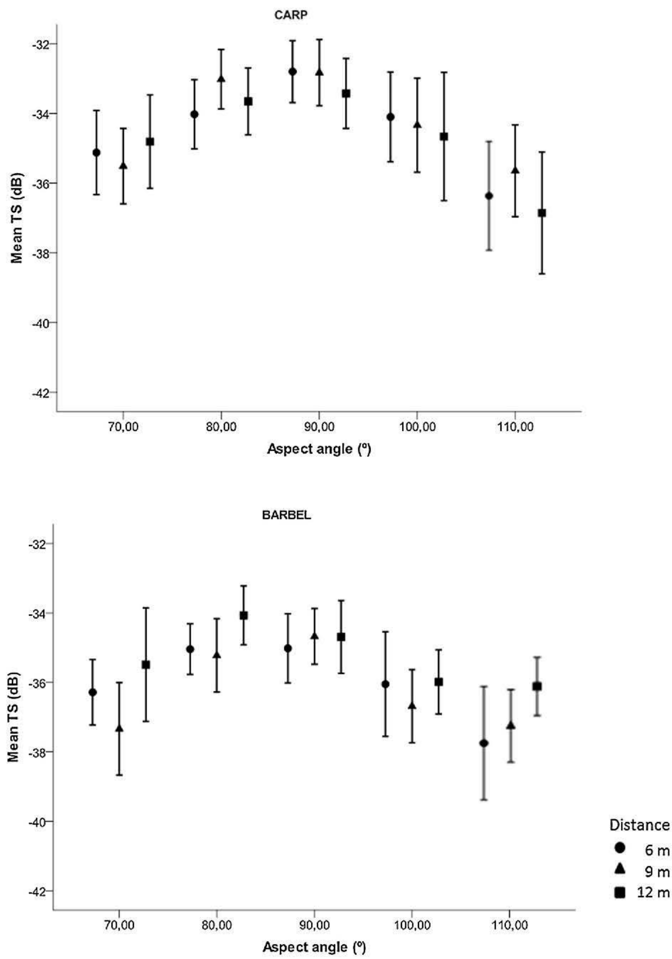

Fig. 5. Lateral mean TS recorded from the studied species at three different distances.

Acknowledgements

This study was carried out without funds thanks to the good will of the fish ecology group. We are grateful to Carlos Granado, Juan Ramon Cid and Carlos Orduna for their invaluable generosity and help with the field work. We are grateful to J.R. Rodríguez-Sánchez for his technical assistance in performing the radiographs. We thank the employees of the Inner Harbour of Seville and the Nautical Club of Seville for their logistical support. Our gratitude goes to Dr. Milan Riha and to the anonymous reviewers for improving this work with their valuable comments and inputs. We thank all those anonymous fishermen for supplying us with large fish. We also thank Cristina Ocaña for her careful proofreading of the English text. Special thanks go to all those who have contributed to this research in any way.

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DISCUSION GENERAL

Studying fish is fundamental to understand the functioning of aquatic ecosystems and to improve their management and conservation. Among the methods available to sample fish communities, hydroacoustics stands out for its many advantages. This technique is widely used and accepted by the scientific community and, consequently, work is currently being undertaken to achieve its standardisation (CEN, 2009). However, horizontal hydroacoustics has only recently started to be applied to study superficial and shallow aquatic systems. Therefore, it is necessary to compare different hydroacoustic systems and study sound behaviour in water under different conditions in order to develop protocols for the use and application of this technique.

In hydroacoustics, the choice of an appropriate TS-length conversion equation is essential to accurately estimate the size and biomass of fish (Boswell and Wilson, 2008; Boswell *et al.*, 2008). In this regard, the comparison conducted in article I between the horizontal conversion equation specifically created for barbel and other previous horizontal conversion equations available for other species (Kubecka and Duncan, 1998; Burwen and Fleischman, 1998; Frouzova and Kubecka, 2004; Frouzova *et al.*, 2005) reveals that the estimated size for barbel varies depending on the equation used and that it is underestimated in all cases. Article III confirms that the differences between equations are mainly dependent on the sampled species, regardless of the methodology employed to create the equations.

Regarding the question of species identification by means of hydroacoustic systems, we agree with Frouzova *et al.* (2005) that horizontal hydroacoustics is not useful to identify species when using the currently available echosounders or with anatomically similar species such as those studied in this work. Nevertheless, due to the significant differences existing between the conversion equations for each species, the use of specific equations is highly recommended. For example, the application of an appropriate conversion equation helps interpret the information about the distribution of fish size more accurately, which is fundamental in studies of energy flow, growth and production. The equations created in this doctoral thesis will allow conducting studies about the behaviour and migration of barbel and carp in shallow and superficial aquatic ecosystems.

Creating conversion equations is not an easy task. Article I introduces several improvements intended to ease the creation of these equations. The current trend when forming equations is to use free-swimming fish since the data collected from them are more similar to those found in natural ecosystems (McClathie *et al.*, 1996; Boswell and Wilson, 2008). Thus far, there is no established or generalised method to calculate the fish's swimming angle (Huse and Ona, 1996; Pedersen *et al.*, 2009). This work presents a new calculation method which has proven to accurately represent the fish's true orientation. The new method includes all of the information backscattered from fish and integrates it in a regression line that summarises their movements. In agreement with previous studies (Pedersen *et al.*, 2009; Jech, 2011; Rodríguez-Sánchez *et al.*, 2015a), we believe that calculating the fish's orientation is fundamental for hydroacoustic studies performed by means of horizontal applications. In this regard, establishing a possible generalised method such as that proposed in this doctoral thesis will contribute to the standardisation of hydroacoustic studies.

Furthermore, we have tried to reduce the amount of time required to create conversion equations. The surface of the main beam available for analysis is theoretically limited to the first -3dB from the acoustic centre (0dB) (Simmonds and MacLennan, 2005). Article I analyses the possible variations that may occur in the TS backscattered from fish when the theoretical surface of the beam is enlarged from -3dB to -5dB. In agreement with the results obtained by Brede *et al.* (1990), the comparisons performed in our study showed that there were no significant differences between the TS values of the tracks located within the central part of the main beam (0º-2.5º from the acoustic axis) and those located outside of the central part (2.5º-4.5º from the acoustic axis). These results confirm that a slight enlargement of the area available for analysis reduces the amount of time required to collect data when creating conversion equations. This enlargement must be implemented very cautiously and it should not be applied when analysing hydroacoustic data recorded from natural systems since this may lead to certain deviations in density and biomass estimates. Therefore, enlarging the area of the main beam available for analysis is only recommended for studies conducted to create conversion equations in conditions similar to those presented in this study.

Furthermore, article I demonstrates that it is not necessary to create conversion equations specific to pulse lengths of 0.128 ms and 0.256 ms when using a Simrad split-beam system at 200 kHz. These results coincide with those obtained in the comparison of dual-beam systems performed by Kubecka (1995). They also coincide with the results obtained by Boswell and Wilson (2008) and Godlewska (2004), where split-beam systems were used with frequencies different from those employed in our study. The results prove that TS measurements remain stable regardless of the pulse length selected for the sampling. Likewise, it has been confirmed that estimates of fish biomass and density obtained from aquatic ecosystems using different pulse lengths are directly comparable.

However, conversion equations cause significant differences depending on the type of beam in the system used (dual or split-beam system). Article II proves that, when TS values are converted, notable differences arise depending on which conversion equations are used, i.e. equations for dual-beam systems or for split-beam systems. These results coincide with those obtained by Traynor and Ehrenberg (1990) or Ehrenberg and Torkelson (1996) in previous experiments. The differences in length estimates are significant and, therefore, so are the differences in biomass estimates. Thus, the equations applied to convert acoustic data must be specific to the type of beam used in order to avoid deviations in biomass estimates.

In addition, the frequency applied also has an effect on the TS, although to a lesser extent than the type of beam. The conversion equations created for the 200 and 430 kHz frequencies were significantly different. In agreement with the results obtained by Kubecka and Duncan (1998), the TS values obtained at 430 kHz were lower than those obtained at 200 kHz in all cases. Furthermore, the fish tracks selected to compare frequencies presented fewer echoes at 430 kHz. These differences could be explained by the directivity property of each frequency. Directivity is higher in systems with higher frequencies (Horne and Clay, 1998), which can cause a reduction of the backscattered energy received and, consequently, a reduction of the TS received. Although Love (1971) recommends using the 430 kHz frequency in samplings at close ranges, this doctoral thesis reveals that the 200 kHz frequency offered better results for the studied species (barbel and carp) when receiving and positioning the acoustic signals.

In spite of these differences, the relationship between frequencies remained stable throughout the different sizes and orientations studied. This implies that the density and biomass estimates obtained by systems operating at different frequencies could be directly comparable by applying a correction factor. This is in agreement with the results obtained in previous studies performed in natural systems where similar frequencies such as 70 or 120 kHz gave the same density estimates (Godlewska *et al.*, 2009). Nevertheless, higher frequencies lead to more pronounced differences, particularly when fish density is high (Wanzenbock *et al.*, 2003; Guillard *et al.*, 2004). The results published in article II coincide with other previously published studies. The conclusion is that different frequencies receive the energy backscattered from fish in different ways, although these differences are not always properly reflected in the density and biomass estimates provided by the systems. Furthermore, this article shows that systems operating at high frequencies may be less useful for horizontal hydroacoustics studies where obtaining accurate information about the fish's swimming angle is fundamental to estimate fish size and biomass. Therefore, we recommend applying a frequency of 200 kHz or lower when measurements of horizontal hydroacoustics are performed at close range.

All these comparisons are important to determine which system should be used in hydroacoustic samplings and to establish protocols for the use of horizontal hydroacoustics, which are necessary in the light of the new European standards (CEN, 2009). In addition, these results help to properly interpret and compare the acoustic data provided by different systems when conducting the intercalibration process as demanded by the WFD.

Finally, this study also focuses on a very important issue for horizontal hydroacoustics that has a direct impact on its applicability to study shallow systems: distance. According to the results presented in article III, horizontal TS values of large fish do not depend on the studied distances (6, 9 and 12 m). In agreement with the studies by Dawson *et al.* (2000) and Knudsen *et al.* (2004), we conclude that the standard formulae to calculate the near-field overestimate the proportion of space occupied by this area. This is why we suggest performing the near-field calculations based on the length of the swim bladder since this organ is responsible for most of the energy backscattered from fish (Foote, 1980; Hazen and Horne, 2003). In addition, the near-field calculated based on the swim bladder is smaller than that calculated based on the total fish length, which indirectly involves an increase in the water volume available for analysis. This confirms that horizontal hydroacoustics is once again a very useful tool to study fish in superficial and shallow systems at close ranges.

Another problem discussed in article III is that of the ensonification of large fish at close ranges, i.e. fish with lengths larger than the width of the acoustic beam. It is to be expected that the TS of a large fish partially ensonified at close range will be different from the TS obtained by the same fish from a greater distance, where the beam width allows for its entire ensonification. Our results showed that the mean TS of large fish ensonified at close range remained stable along the studied distances. Accordingly, if the swim bladder is included in the main beam at a given distance, the sound coming from the fish is presumed not to vary even when its body is not entirely ensonified.

The results obtained in this doctoral thesis highlight the usefulness and accuracy of density and biomass estimates obtained by means of horizontal hydroacoustics. They clarify previous speculations and represent a step forward in the understanding of sound behaviour for the detection of fish in shallow systems. We believe that horizontal hydroacoustics has a promising future for the studies of fish. In this regard, this technique could be very useful for routine management tasks of shallow systems such as the fish ponds used in aquaculture.

PROSPECTS OF THE IMPLEMENTATION OF HORIZONTAL HYDROACOUSTICS

Horizontal hydroacoustics presents very interesting prospects in the field of fish ecology. There are many shallow aquatic systems in which performing samplings to obtain absolute quantitative values is quite difficult. Horizontal hydroacoustics may be the perfect complement for traditional fishing gears. This technique could reduce the biases derived from the selectivity effect associated with traditional fishing methods when performing studies about fish populations in shallow ecosystems. In studies of deep bodies of water such as reservoirs and lakes, horizontal hydroacoustics can also complement the estimates obtained by means of vertical hydroacoustics since it would provide information about coastal areas where vertical applications are not effective. Combining both techniques (vertical and horizontal) would be the most advisable action since the entire ecosystem could be studied and estimates would include both fish that live in deep areas and those living in shallow and superficial zones.

Horizontal hydroacoustics is also emerging as a very useful technique for fish migration studies that can replace traditional capture and recapture techniques. In fact, hydroacoustics started to be used a few years ago in migration studies of salmonids in rivers from North America and Europe (Steig and Iverson, 1998; Ransom *et al.*, 1996). Nevertheless, when hydroacoustic samplings are performed in very shallow areas where certain obstacles complicate fish detection (submerged vegetation, reverberation of surface and bottom, etc.), it is necessary to conduct even more surveys and calibrations in order to properly interpret the results. In this regard, the technique has yet to be improved and its possible limitations must be identified.

Likewise, horizontal hydroacoustics could be used for the management of shallow systems used in aquaculture for the breeding of species. In aquaculture, it is necessary to conduct periodic censuses to determine the size and number of fish. The use of acoustic methods could be a way to avoid direct manipulation and the stress that extractive samplings entail for fish. In fact, the methodological foundation implemented in this doctoral thesis is currently being used to develop new practices suitable for the management of aquaculture ponds. This study has the objective of creating a sampling protocol to determine the number and size of fish from fish ponds located on land in order to include hydroacoustic techniques in the routine management of this kind of fish farm.

To sum up, horizontal hydroacoustics has only recently started to be used to analyse shallow systems. It is necessary to conduct new studies that help develop this technique and that provide tools for its proper understanding and interpretation. In this regard, this doctoral thesis significantly contributes to the development and application of horizontal hydroacoustics. We have studied the effect that some of the most important parameters have on the conversion of acoustic results. These parameters include the studied species, the frequency applied, the system used to collect data and the pulse length applied. This research has revealed that this technique offers excellent conditions to be successfully implemented in fish studies conducted in superficial and shallow ecosystems.

This work highlights the importance of using TS-length conversion equations specific to the species and systems used (dual-beam or split-beam systems). We have also found that high frequencies (430 kHz) can result in being less appropriate to study shallow ecosystems. The 200 kHz frequency offers better results when positioning echoes from fish and, thus, determining the fish's swimming angle is more accurate, which prevents deviations in size and biomass estimates. In addition, this study shows that horizontal hydroacoustics works properly within the first metres of sampling, which allows obtaining stable sound measurements. This is very encouraging since the information obtained by means of this technique is collected within the first metres of sampling.

We would like to highlight that this kind of study is completely necessary to move forward and improve this technique. On the one hand, it helps to understand the behaviour of sound when applied to fish detection, which in turn leads to scientific tools to justify the selection of systems. On the other hand, they provide useful information for the intercalibration processes demanded by the Water Framework Directive.

Horizontal hydroacoustics presents new prospects of use in studies of fish populations in aquatic ecosystems. It can be highly useful to study the migration of species in riverside systems. Furthermore, the combination of horizontal and vertical hydroacoustics results in more complete density estimates that are closer to the true values found in aquatic ecosystems. Finally, horizontal hydroacoustics could be useful for the management of fish populations from fish ponds and other production systems where knowing the abundance of fish in real time is of vital importance.

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CONCLUSIONS

- Horizontal acoustic signals (TS) of barbel and carp vary depending on the fish's orientation with respect to the acoustic beam. Therefore, TS-length conversion equations must include information about the fish's swimming angle.

- Using species-specific equations is fundamental to obtain accurate biomass estimates since TS-length conversion equations for barbel and carp are significantly different.

- It is recommended the generalised application of the method developed to calculate the fish's swimming angle as it represents the fish's real orientation and it integrates all of the acoustic information reflected in their movements.

- When creating conversion equations under conditions similar to those presented in this study, it is possible to enlarge the surface of the main beam up to -5dB from the acoustic centre without causing variations in the TS of the ensonified fish.

- It is not necessary to create conversion equations specific to pulse lengths of 0.128 ms and 0.256 ms when using Simrad split-beam systems at 200 kHz.

- Biomass results calculated on the basis of hydroacoustic data are more accurate when the conversion equations applied are specific to the type of beam (dual beam or split beam) and to the frequency used.

- The 200 kHz frequency offers better results when receiving and positioning the acoustic signals. Hence, its use is recommended in hydroacoustic studies about barbel and carp conducted in shallow systems.

- The standard formula to calculate the near-field overestimates the proportion of space occupied by this area. It is recommended performing the near-field calculations based on the length of the swim bladder since this organ is responsible for most of the energy backscattered from fish.

- In horizontal hydroacoustics, the mean TS values of large fish ensonified at close range remain stable within the first metres of ensonification.

VERSIÓN EN CASTELLANO

INTRODUCCION

1.- La hidroacústica como técnica para el estudio de la ecología de peces

Estudiar y entender la ecología de los peces no es tarea fácil ya que éstos viven en un medio ajeno al nuestro y el acceso a la información resulta complejo. Sin embargo, los peces son componentes muy importantes del ecosistema acuático y su estudio es fundamental para el conocimiento del mismo. El reconocimiento de su importancia es relativamente reciente ya que hasta la segunda mitad del siglo XX este grupo de vertebrados había sido el gran olvidado de la ecología. La visión clásica del funcionamiento del ecosistema acuático excluía su participación como agentes reguladores. El ecosistema acuático era considerado una máquina funcional donde los productores primarios dirigían su energía hacia los niveles superiores de depredadores (control bottom-up). Dentro de este concepto, los peces no tenían una función importante en la regulación del sistema y su estudio era escaso y, en general, de tipo descriptivo (Granado-Lorencio, 2001; Encina *et al.*, 2006). No es hasta el trabajo de Brooks and Dodson (1965), cuando los peces cobran protagonismo en el ecosistema. Con ellos aparece una nueva teoría que sitúa al grupo de los peces en el centro del funcionamiento del ecosistema (control top-down). Posteriormente, el trabajo de Northcote (1988) resume la función de los peces en el ecosistema integrando la clásica visión bottom-up y la nueva visión top-down. Desde esta perspectiva, la depredación controla la estructura de la comunidad mientras que la competencia y la disponibilidad de recursos limita la máxima producción de cada nivel trófico (Mills and Forney, 1988). Este concepto de cascada trófica ensambla los principios de la limnología y de la ecología pesquera y establece que interviniendo los “stocks” de los peces en el ambiente acuático se podrían controlar los niveles inferiores de la cadena trófica (Carpenter *et al.*, 1985). A partir de estos trabajos crece enormemente el interés por el estudio de las comunidades de peces. Para su estudio deben analizarse diversos procesos y aspectos de su biología para tener una visión holística del sistema. Necesitamos conocer las asociaciones de peces, su composición y regulación en el tiempo; su abundancia y el papel que estos juegan en el flujo de energía; la ocupación de los hábitats acuáticos y las relaciones de los peces con otros componentes del sistema (presas y predadores), sin olvidar los temas relacionados con la conservación o con el manejo y gestión de los ecosistemas acuáticos.

Existen diversos métodos para el estudio de las poblaciones de peces que podemos englobar en dos grandes grupos: métodos dependientes de captura y métodos independientes de captura (Lucas and Baras, 2000). Entre los métodos dependientes de captura encontramos las redes agalleras, redes de arrastre, redes de cerco, pesca eléctrica, etc. Estas técnicas ofrecen una escasa cobertura de muestreo y no proporcionan información sobre los valores absolutos de densidad y biomasa de las comunidades de peces estudiadas. Además, debido a la distribución poco homogénea de los peces y a la influencia de aspectos etológicos y de aspectos relacionados con la selectividad de las artes de pesca, la información obtenida con estas técnicas puede no ser suficientemente representativa del ecosistema (Emmrich *et al.*, 2012). Entre los métodos de pesca independientes de captura destaca la técnica hidroacústica, porque permite cuantificar la distribución y abundancia de peces de una forma no selectiva. Es además muy útil para estudios de comportamiento y migración de especies tanto en los sistemas de agua dulce como en los sistemas marinos (Guillard 1998; Lucas y Baras 2000; Simmonds y MacLennan, 2005) y al contrario que las técnicas de estudio por imagen, es efectiva incluso en ecosistemas donde la visibilidad es reducida. Por ello, numerosos estudios confirman su idoneidad para el estudio de los ecosistemas acuáticos y renunciar a su uso resulta difícilmente justificable en cuanto a la relación coste-rendimiento (Godlewska, 2004, Monteoliva y Schneider, 2005; Kubecka *et al.*, 2009).

Entre las ventajas que presenta esta técnica destacamos que nos permite estudiar grandes superficies de agua en poco tiempo y analizar a la vez la información contenida en los distintos compartimentos del ecosistema (Brandt, 1996; Wanzeböck *et al.*, 2003; Godlewska., 2004; Winfield *et al.*, 2007). Además, es una técnica no letal que no interfiere directamente sobre los organismos del sistema que estudia. El inconveniente más importante que presenta la hidroacústica es que no es capaz de identificar especies por lo que debe utilizarse en combinación con otras técnicas para conseguir esta información. No obstante, numerosos estudios recomiendan el uso combinado de varias técnicas de muestreo para lograr una visión completa del ecosistema acuático. La aplicación de técnicas múltiples minimizan el efecto de la selectividad de las técnicas tradicionales y complementan las informaciones obtenidas con cada técnica por separado, proporcionando finalmente mejores resultados (Prchalova *et al.*, 2009; Winfield *et al.*, 2009; Harrison *et al.*, 2010; Emmrich *et al.*, 2012; Kubecka *et al.*, 2012).

La técnica hidroacústica utiliza el sonido para el estudio de los ecosistemas acuáticos. Durante mucho tiempo, los investigadores han sentido una gran fascinación por el sonido y el modo en que éste se desplaza por el agua. Ya en 1490, Leonardo Da Vinci observó: "Si detiene su barco y coloca la punta de un tubo de gran longitud en el agua y el otro extremo lo acerca a su oído, podrá escuchar barcos que se encuentren a gran distancia". En 1826 Daniel Colladon, un físico suizo, y Charles Sturm, un matemático francés, midieron de forma precisa la velocidad del sonido en el agua (Fig.1). Con la ayuda de un tubo largo para escuchar debajo del agua (como había sugerido Da Vinci), consiguieron registrar cuánto tiempo tardaba el sonido de una campana sumergida en recorrer todo el Lago Lemán. El resultado fue 1.435 metros (1.569 yardas) por segundo en agua a 1,8 ºC (35º F), sólo 3 metros por segundo menos de la velocidad aceptada hoy día. Lo que demostraron estos investigadores fue que el agua, ya sea dulce o salada, es un medio excelente para el sonido, transmitiéndose casi cinco veces más rápido que en el aire (National Academy of Science, 2003) Washington D.C. (http://www.nationalacademies.org).

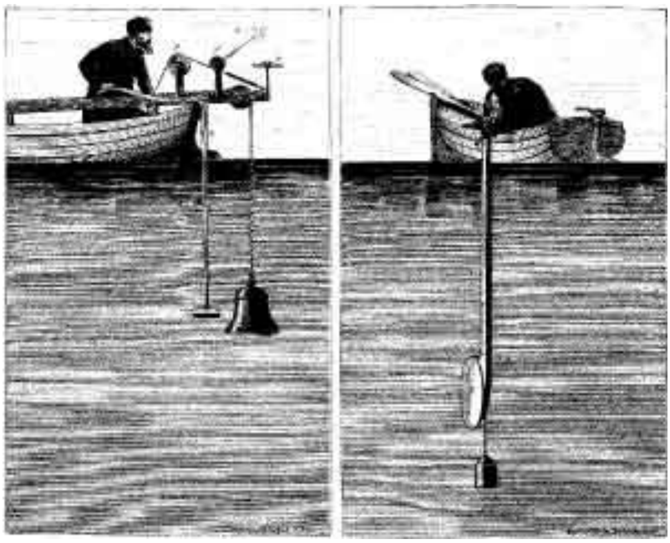

Figura 1 Charles Sturm (izquierda) y Daniel Colladon (derecha) midiendo por primera vez la velocidad del sonido en el agua (ilustración propiedad de la Sociedad Acústica de Norteamérica).

No es hasta principios del siglo XX, cuando la hidroacústica encuentra una aplicación práctica como resultado de los avances tecnológicos. El descubrimiento de la piezoelectricidad por Jacques y Pierre Curie en 1880 fue fundamental para el desarrollo del transmisor piezo-eléctrico en 1917, que dio paso a las primeras experiencias hidroacústicas. En los años 20 se utilizó por primera vez el término echosounding, en referencia a la técnica utilizada para medir la profundidad de la columna de agua y unos años más tarde, en 1929, Kimura realizó la primera experiencia satisfactoria en la detección de peces (Simmonds y MacLennan, 2005). En las décadas de los 70 y 80 con los estudios de Love (1977) o Foote (1980) Foote *et al.* (1987) se determina el sonido devuelto por los peces y se establecen ecuaciones que relacionan la energía devuelta de un pez con su tamaño. Estas nuevas relaciones se incorporan a las rutinas de muestreo hidroacústico dando resultados esperanzadores en las estimas de densidad y biomasa de peces en los ecosistemas. Desde entonces hasta ahora, intensas investigaciones teóricas y experimentales han permitido un mejor conocimiento y aplicación de estas técnicas.

En la actualidad, el comité de normalización europeo (CEN) ha comenzado la elaboración de una norma estándar para su uso (CEN, 2009). Para ayudar al desarrollo de estas normativas la hidroacústica necesita resolver ciertas cuestiones relacionadas con su funcionamiento. El objetivo de la normalización no es otro que proporcionar herramientas que permitan la comparación de los datos obtenidos en diferentes cuerpos de agua utilizando sistemas diferentes.

Por otra parte, la puesta en práctica de la Directiva Marco del Agua (DMA) Europea (2000/60/CE) requiere que los estados europeos realicen una evaluación de todas sus superficies acuáticas y se aseguren de su buen estado ecológico para el 2015. La directiva europea requiere que los resultados de los trabajos realizados en cualquier ecosistema acuático dentro de la Unión Europea sean directamente comparables. En general, los datos difieren en cuanto a las técnicas de muestreo utilizadas o en los sistemas aplicados. Estas diferencias metodológicas conducen a importantes dificultades en la interpretación de los datos y dificultan su comparación directa (inter-calibración). En el proceso de inter-calibración requerido por la DMA la hidroacústica necesita estudiar estas diferencias para definir y unificar los criterios internacionales a utilizar en su uso combinado con otras técnicas. En este sentido, aunque existen algunas comparaciones previas (Wanzeböck *et al.*, 2003, Guillard *et al.*, 2004; Guillard y Vergès, 2007; Rakowitz *et al.*, 2008; Godlewska *et al.*, 2009), son numerosos y diversos los temas que quedan por investigar. Se necesitan por tanto, un mayor número de experiencias para llegar a conseguir la normalización de esta metodología. Trabajos como los que aquí se exponen tratan de resolver algunos de los problemas planteados con el uso y aplicación de la técnica hidroacústica horizontal y realiza comparaciones entre sistemas y frecuencias a fin de facilitar la comparación de datos hidroacústicos obtenidos con diferentes aparatos, facilitando así la inter-calibración de sistemas.

2.- ¿Cómo funciona la hidroacústica?

La hidroacústica es una técnica que aplica el sonido y sus propiedades para permitir la detección remota y posicionamiento de los objetos sumergidos en el ecosistema acuático. Para conseguir la información utiliza un aparato llamado ecosonda, que funciona como transmisor y receptor de señales sonoras. Este aparato, emite ondas sonoras que viajan en el agua chocando con todos los organismos y partículas que encuentra a su paso. Cada uno de los obstáculos con que tropiezan las ondas sonoras refleja a su vez un eco de vuelta. Todos los ecos reflejados son recibidos por el receptor de la ecosonda y el programa de adquisición de datos los traduce en una imagen (ecograma) que representa el ecosistema subacuático (Fig.2). Los peces, como blancos acústicos devuelven también un eco y su reflectividad se resume en un parámetro conocido como retrodispersión de la sección transversal (σ_{bs}), que es fundamentalmente el tamaño acústico del pez. Este parámetro suele expresarse en su dominio logarítmico y conocido como fuerza del blanco, comúnmente conocido por su traducción inglesa Target Strength o TS (en dB relativo a 1 m^2) (Sunardi *et al.*, 2009).

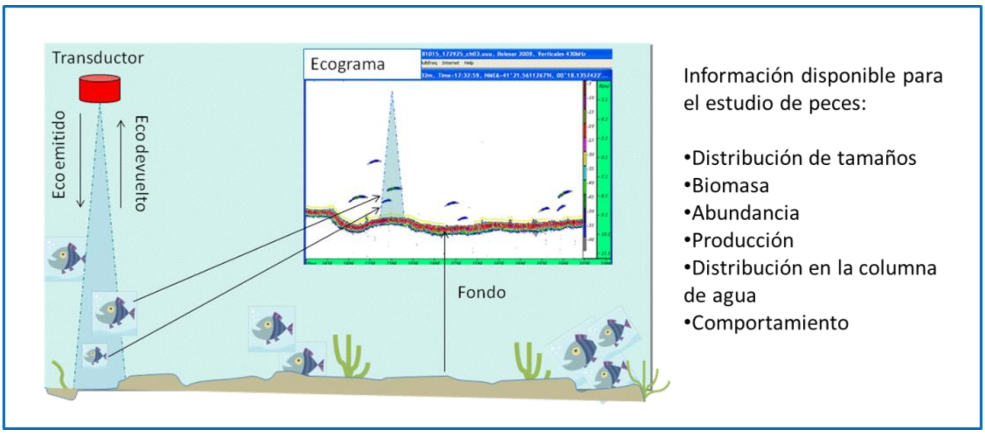

Figura 2. Esquema del funcionamiento de una ecosonda y resultados del muestreo.

La llave para la correcta interpretación de los datos de un muestreo hidroacústico es, por tanto, el valor de la fuerza del blanco (TS) que devuelven los peces. Conociendo este valor, se construyen ecuaciones de conversión que permiten traducir el sonido devuelto por un pez (TS) en parámetros biológicos medibles como su longitud o peso. La aplicación de estas ecuaciones en el análisis de la información recogida en un muestreo hidroacústico permite calcular el tamaño, el número y la posición de los peces en el ecosistema (Lucas y Baras, 2000).

El valor de la fuerza del blanco (TS) depende en general de parámetros morfológicos del pez como su tamaño y sus características anatómicas, el tipo y tamaño de la vejiga natatoria que presenta, la especie, etc. También depende de parámetros ajenos a la naturaleza del pez como son la posición del pez en el haz acústico, la orientación de natación del pez, el sistema de insonificación utilizado (haz simple, doble o partido), la frecuencia aplicada, etc. (Horne y Clay, 1998; Sawada *et al.*, 2002; Hazen y Horne, 2003; Simmonds y MacLennan, 2005; Pedersen *et al.*, 2009; Jech, 2011). Todas estas características deben tenerse en consideración cuando se construyen ecuaciones de conversión, de modo que en la mayoría de los casos, estas ecuaciones serán específicas para los parámetros anteriormente enunciados.

Aunque existen ecuaciones de conversión para algunas especies de peces de agua dulce, tanto en posiciones laterales como para otros aspectos (Borisenko *et al.*, 1989; Burwen y Fleischman, 1998; Lilja *et al.*, 2000; Frouzova y Kubecka, 2004; Knudsen *et al.*, 2004; Frouzova *et al.*, 2005; Boswell y Wilson, 2008), no son suficientes para cubrir las necesidades que la hidroacústica presenta debido a la amplia gama de especies, frecuencias y tipos de ecosonda utilizadas (Lucas y Baras, 2000).

3.- La hidroacústica horizontal

Tradicionalmente, la hidroacústica ha sido aplicada con el haz acústico orientado verticalmente (perpendicular a la superficie del agua) en sistemas marinos (Simmonds y MacLennan, 2005. En estos ambientes, la técnica ha mejorado y se ha desarrollado extensamente, demostrando ser una técnica de muestreo útil para los estudios de ecología de peces (Lucas y Baras, 2000). Poco a poco, su uso se ha extendido a los ecosistemas de agua dulce (Brandt, 1996) y en su aplicación se han encontrado problemas diferentes a los planteados en el medio marino. Entre ellos destaca que el muestreo vertical encuentra limitaciones cuando los sistemas son poco profundos. Los estudios de Kubecka y Wittingerova (1998) y Knudsen y Saegrov (2002) alertan de la posibilidad de una subestimación de la densidad de los ecosistemas debida a la imposibilidad de contar los peces que se encuentran cerca de la superficie o en hábitats someros. Ciertamente, en las capas superficiales, el haz acústico es tan pequeño que el muestreo vertical no es suficiente para cubrir esas áreas. La técnica encuentra limitaciones para la interpretación de los datos en estas zonas debido a lo que se conoce como zona-ciega hidroacústica. Para solventar esta limitación ambos trabajos sugieren completar la información proporcionada en muestreos verticales con datos de hidroacústica horizontal (con el haz orientado paralelo a la superficie del agua).

Las aplicaciones horizontales se emplean generalmente para estudiar los ecosistemas poco profundos, tales como sistemas fluviales o capas superficiales en grandes sistemas de agua profundos, ya que un gran número de los peces utilizan estos hábitats superficiales como refugio, área de alimentación, etc. (Encina *et al.*, 2006; Kubecka *et al.*, 2012). A diferencia de la aplicación vertical, la aplicación horizontal está menos desarrollada y necesita de estudios que resuelvan las cuestiones que van apareciendo conforme su uso se hace más extendido.

La hidroacústica horizontal necesita de ecuaciones específicas, diferentes a las realizadas en aplicación vertical, que incorporen información sobre la orientación de natación del pez (Hazen y Horne, 2003; Simmonds y Maclennan, 2005; Pedersen *et al.*, 2009; Jech 2011, Rodríguez-Sánchez *et al.*, 2015a,b). La carencia de ecuaciones presentada en la aplicación vertical se agrava en la aplicación horizontal ya que su uso es muy reciente y sólo existen conversiones para algunas especies y sistemas hidroacústicos (Burwen y Fleischman, 1998; Lilja *et al.*, 2000; Frouzova y Kubecka, 2004; Frouzova *et al.*, 2005; Boswell y Wilson, 2008).

Para el desarrollo experimental de las ecuaciones TS, lo ideal es contar con peces con natación libre ya que los resultados estarán más próximos a los valores que podemos encontrar en la naturaleza (Simmonds y MacLennan, 2005). Sin embargo, la utilización de peces con natación libre presenta ciertos problemas asociados con la determinación del ángulo de natación del pez y con el tiempo empleado para conseguir datos de calidad. Actualmente no existen métodos establecidos para calcular el ángulo de natación del pez. Algunos autores utilizan la información que le proporcionan medios visuales como la fotografía (Huse y Ona, 1996), otros utilizan las posiciones XY de los ecos de la trayectoria de natación y la velocidad de movimiento del pez (Pederson *et al.*, 2009). Balk y Lindem (2011) facilitan diferentes métodos para el cálculo de natación del ángulo según las necesidades del investigador, pero no existen métodos sencillos y consensuados que permitan el cálculo del ángulo de natación del pez en su orientación horizontal. Además, el tiempo que se necesita para la elaboración de las ecuaciones de conversión puede llegar a ser un factor limitante. La adquisición de datos acústicos con peces en natación libre es laboriosa y la selección de trayectorias lineales debe hacerse manualmente (Simmonds y MacLennan, 2005) lo que puede dilatar enormemente la obtención de datos adecuados.

En el artículo I "Horizontal target strength of *Luciobarbus sp*. in *ex-situ* experiments: Testing differences by aspect angle, pulse length and beam position" se presentan las ecuaciones de conversión horizontal para el género barbo realizadas con un sistema de haz partido trabajando a 200 kHz de frecuencia. Este género es uno de los más importantes en las asociaciones piscícolas de la Península Ibérica. Además, está ampliamente distribuido por África, Asia y Europa siendo, en numerosos ecosistemas, componente dominante de la comunidad de ciprínidos. A pesar de su amplia distribución geográfica, no existe información acústica referente a este género. Por tanto, su estudio acústico es urgente para la mejora de los resultados de los estudios ecológicos realizados en estos sistemas.

En este artículo, se presentan además los resultados correspondientes al análisis de los problemas relativos a la construcción de las ecuaciones de conversión horizontal, a fin de buscar soluciones y facilitar su desarrollo. Para la determinación del ángulo de natación del pez, se ha desarrollado un nuevo método que calcula su orientación integrando en una recta de regresión toda la energía devuelta por el pez en movimiento. Para asegurar la bondad de la aproximación, los resultados han sido contrastados con grabaciones de video simultáneas realizadas durante la insonificación. En este mismo experimento se estudia también el comportamiento del TS devuelto en diferentes posiciones del haz acústico, con el objetivo de poder ampliar el área útil de adquisición de datos y de reducir, por tanto, el tiempo necesario para la adquisición y la obtención de las ecuaciones de conversión.

Además de la ecuación utilizada para la conversión de los datos hidroacústicos existe otro parámetro que se selecciona al inicio del muestreo y que determina la capacidad de resolución del mismo: la longitud de pulso. Este parámetro determina el tiempo que dura la emisión de los trenes de onda e influye sobre la capacidad de discriminación del sistema cuando los peces se encuentran próximos entre sí. Si la longitud de pulso es demasiado larga, el tren de ondas emitido podría englobar dos o más peces a la vez y el sistema los interpretaría como un único pez. En ese caso, la selección de una longitud de pulso más corta permitiría que cada pez fuera englobado por un tren de ondas diferente y el sistema los interpretaría como peces individuales (Figura 3). Por eso, cuando la densidad de peces es alta se suelen seleccionar longitudes de pulso bajas, porque éstas permiten una mejor identificación de los peces individuales. Estudios previos han determinado que utilizando longitudes de pulso cortas (0.1-0.4 ms) no parecen existir diferencias en el TS devuelto por los peces (Kubecka, 1995; Boswell y Wilson, 2008; Godlewska *et al.*, 2011).

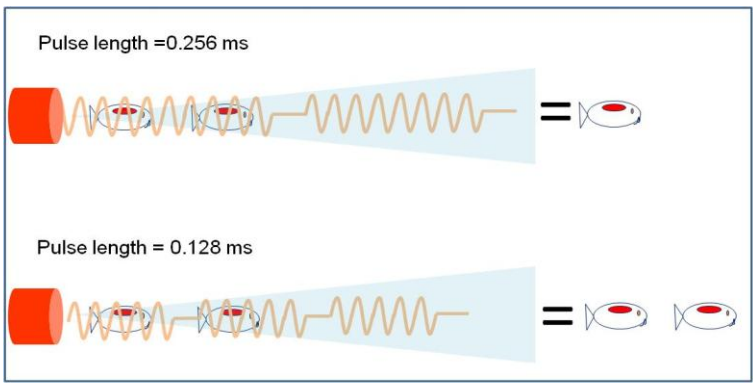

Figura 3. Esquema gráfico del efecto de la longitud de pulso sobre la detección de peces adyacentes

Los efectos de la longitud de pulso se han estudiado sobre sistemas y frecuencias diferentes a los aplicados en este trabajo por lo que en el artículo nos planteamos la posibilidad de contrastar esta información con nuestro sistema de haz partido de 200 kHz de frecuencia. Para realizar la comparación, se estudiaron los valores de TS para *Luciobarbus sp*. utilizando dos longitudes de pulso diferentes.

Con el uso y aplicación de la hidroacústica horizontal, aparecen nuevas incertidumbres relacionadas con la adquisición de datos. Dada la variedad de ecosondas existentes (haz simple, doble o partido), frecuencias y parámetros de adquisición, la elección del sistema correcto y de los parámetros adecuados para optimizar el muestro acústico dependerá de las características del ecosistema acuático, de los organismos que queremos estudiar y del conocimiento que tengamos sobre cómo funcionan cada una de las herramientas que la hidroacústica proporciona para el estudio del ecosistema.

En relación a la elección del sistema a utilizar, la hidroacústica horizontal necesita información referente a la posición de los peces por lo que necesita sistemas que utilicen dos o más haces. Los sistemas de haz doble y partido son útiles para este tipo de muestreos aunque funcionan de forma diferente. Los sistemas de haz doble utilizan transductores que emiten el sonido en dos haces concéntricos que funcionan alternativamente permitiendo corregir las señales acústicas (ecos) por distancia y posición. Los sistemas de haz partido emiten la señal acústica de una vez pero para la recepción de las señales acústica (ecos) presentan el haz dividido en cuatro cuadrantes. La comparación de la información sonora recibida por cada cuadrante permite la identificación y posicionamiento de blancos individuales en un espacio tridimensional dentro del haz sonoro. Ambos sistemas son útiles en sistemas someros pero existen algunas cuestiones que deberían ser estudiadas previamente como, por ejemplo, ¿Reciben la misma información acústica de los peces? ¿Podemos utilizar ecuaciones desarrolladas para sistemas de haz doble en sistemas de haz partido sin modificar los resultados de biomasa y densidad?

Trabajos previos han estudiado los resultados acústicos obtenidos por sistemas de haz doble y partido. Traynor y Ehrenberg (1990) registraron medidas de TS procedentes de una bola de calibración utilizando sistemas de haz doble y partido. Encontrando que los TS obtenidos con sistemas de haz partido eran menos variables que los obtenidos con sistemas de haz doble. Posteriormente, Ehrenberg y Torkelson (1996) utilizaron resultados teóricos y compararon el efecto que el ruido producía en ambos sistemas. Observaron que los sistemas de haz partido resultaban menos afectados por el incremento de sonido y por tanto eran más adecuados para estudios desarrollados en sistemas ruidosos. Los resultados de estos trabajos, realizados sobre modelos teóricos y blancos de sonido conocido, responden a la primera pregunta y demuestran que ambos sistemas (haz doble vs. haz partido) no reciben del mismo modo la información acústica de los blancos. Por tanto, ambos sistemas presentaran con toda probabilidad diferencias en la detección de los peces.

En el artículo II: “Horizontal target strength of *Cyprinus carpio* using 200 kHz and 430 kHz split beam systems” se han desarrollado las ecuaciones de conversión horizontal para la especie *Cyprinus carpio* (carpa común) con dos frecuencias diferentes. La carpa común es también una especie importante en las asociaciones ícticas de los sistemas de agua dulce de toda Europa. Es nativa de Asia aunque puede encontrarse en casi todo el mundo a excepción de Oriente medio y los polos (Kottelat y Freyhof, 2007). Esta especie contribuye significativamente a la biomasa total de los sistemas acuáticos dado su gran tamaño y abundancia, por lo que su estudio acústico mejorará, sin duda, los resultados de las prospecciones acústicas de estos ecosistemas.

Además de la construcción de estas ecuaciones, en este artículo se ha trabajado para dar respuesta a la pregunta sobre si se pueden utilizar las ecuaciones desarrolladas para sistemas de haz doble en trabajos que utilicen sistemas de haz partido sin modificar los resultados. Para ello se han comparado las ecuaciones de conversión horizontal de carpa común obtenidas con dos sistemas de haz partido de frecuencias 200 y 430 kHz con las desarrolladas por Kubecka y Duncan (1998) para la misma especie y las mismas frecuencias utilizando sistemas de haz doble.

Otra de las cuestiones que los investigadores se plantean a la hora de elegir el sistema acústico adecuado para el estudio de las poblaciones de peces es la frecuencia a utilizar. Obtener unos buenos resultados de las estimas de densidad y biomasa dependerán por una parte, de la aplicación de una ecuación de conversión adecuada y por otra, de la correcta elección de la frecuencia de muestreo.

Las frecuencias altas, en comparación con sistemas de baja frecuencia, proporcionan una mayor resolución del sistema, aunque no obtienen buenos resultados en ecosistemas con alta densidad de peces o ambientes muy ruidosos (Simmonds y Maclennan, 2005). Degan y Wilson (1995) aconsejan para la detección de peces el uso de frecuencias de entre 120-200 kHz, aunque otros estudios han aplicado frecuencias mayores para estudiar el sonido de los peces o para estimar la densidad de las poblaciones de peces (Kubecka *et al.*, 1994; Beauchamp *et al.*, 1997; Kubecka y Duncan, 1998). En hidroacústica horizontal no sólo necesitamos que la frecuencia aplicada reconozca peces individuales. Además, la frecuencia seleccionada debe posicionar con exactitud los ecos devueltos, ya que el cálculo del ángulo de natación de los peces es fundamental para la interpretación de los datos acústicos. Las comparaciones entre frecuencias son escasas (Wanzenböck *et al.*, 2003; Guillard *et al.*, 2004; Godlewska *et al.*, 2009) y en orientación horizontal no existen estudios que comparen la capacidad que presentan diferentes frecuencias a la hora de posicionar los ecos. Estos estudios son necesarios porque por una parte, desarrollan nuestro conocimiento sobre el funcionamiento de las diferentes frecuencias y, por otra parte, nos proporcionan herramientas para determinar cuál de ellas es más adecuada para nuestro estudio.

A la luz de estas necesidades, en el artículo II se analizaron las estimas de posición ofrecidas por las frecuencias de 200 y 430 kHz utilizando trayectorias acústicas de peces insonificados simultáneamente. El objetivo de este análisis fue determinar qué frecuencia posicionaba con mayor precisión las trayectorias de peces en movimiento en sistemas poco profundos.

En el análisis de los datos hidroacústicos obtenidos con la aplicación horizontal, la distancia útil de muestreo es otro factor a tener en cuenta por varias razones. En primer lugar, al estudiar sistemas superficiales y someros, la reverberación producida por la superficie y el fondo limitan el rango útil para el análisis (Mulligan, 2000). La relación entre la señal del pez y el ruido (signal to noise ratio, SNR) es el parámetro acústico que cuantifica este efecto. Así, a una distancia determinada del transductor, el efecto de las reverberaciones puede ser tan grande que las señales de los peces sean indistinguibles (SNR baja), limitándose así la distancia máxima de análisis.

En segundo lugar, la teoría nos habla de otro efecto que limita la distancia mínima de análisis, es decir, la distancia que se debe guardar desde el transductor hasta el pez para que la toma de datos sea correcta. Teóricamente, las mediciones de TS no se deben tomar en la zona conocida como campo cercano (Simmonds y MacLennan, 2005). Esta zona se localiza adyacente a la superficie del pez y en ella el sonido sufre múltiples oscilaciones, haciendo que las mediciones de TS sean inestables (Dawson *et al.*, 2000). El campo cercano se calcula a partir de la longitud del pez y está directamente relacionado con este parámetro. Por ejemplo un pez de 400 mm tiene un campo cercano de aproximadamente 5,5m, lo que significa que teóricamente deberíamos comenzar el análisis acústico guardando una distancia mínima con el transductor de 5,5 m, limitando así el volumen útil del muestreo (Figura 4).

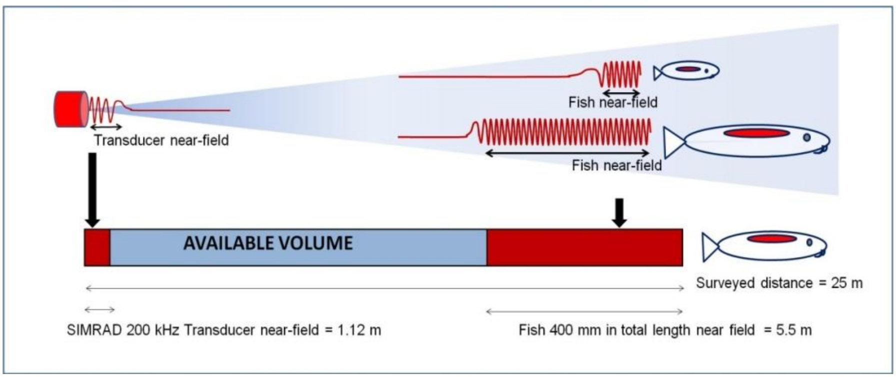

Figura 4: Efecto del campo cercano. En el gráfico superior están representados los campos cercanos del transductor y de dos peces de diferente tamaño. En el gráfico inferior se representa el volumen insonificado (rectángulo negro) señalándose en rojo los espacios ocupados por el campo cercano del transductor y de un pez de longitud determinada y en azul el volumen teórico disponible para el análisis.

Una última cuestión relacionada con la distancia tiene que ver con la insonificación de peces grandes cerca del transductor. La mayor parte de la señal acústica emitida por el transductor se dispersa cónicamente por lo que un pez grande cerca del transductor puede quedar sólo parcialmente insonificado y su sonido puede ser diferente al presentado por ese mismo pez insonificado a una mayor distancia, donde quede englobado completamente. Tener en cuenta estas limitaciones (distancia máxima y mínima desde el transductor) podrían, en algunos casos, invalidar la información de los muestreos hidroacústicos realizados con orientación horizontal.

El artículo III presentado en esta tesis: "Do close range measurements affect the target strength (TS) of fish in horizontal beaming hydroacoustic? trata de resolver las cuestiones planteadas en relación a la distancia. Su objetivo es determinar en los primeros metros de insonificación (6, 9 y 12 metros) el rango donde se producen medidas estables de TS en los muestreos realizados con hidroacústica horizontal.

Los resultados obtenidos en esta tesis doctoral pretenden mejorar el uso de las técnicas hidroacústicas en su aplicación horizontal y, con ello, su utilidad como herramienta en el estudio de las poblaciones de peces de los ecosistemas acuáticos epicontinentales. Con las nuevas ecuaciones desarrolladas se mejorarán los resultados de los estudios donde las especies estudiadas sean dominantes en la asociación piscícola, como, por ejemplo, en la mayor parte de los embalses de la Península Ibérica. El conocimiento del comportamiento del sonido en las situaciones estudiadas nos permitirá tener criterios científicos para realizar una buena selección de las herramientas y sistemas a utilizar en función de las características del ecosistema estudiado. Además nos permitirá realizar una mejor interpretación de los datos acústicos obtenidos con sistemas diferentes. Por último, el estudio referente al comportamiento del sonido con la distancia confirma la utilidad y aplicabilidad de la hidroacústica utilizada para la investigación de las comunidades de peces en ecosistemas someros, proporcionándonos información útil para determinar la distancia idónea para la toma de datos y su posterior análisis.

RESULTADOS:

Esta tesis está formada por tres artículos, dos de ellos actualmente publicados (artículo I y III) y el tercero enviado a una revista científica internacional (artículo II). En el texto nos referiremos a ellos por sus números romanos.

ARTÍCULO I

Horizontal target strength of *Luciobarbus sp.* in *ex situ* experiments: testing differences by aspect angle, pulse length and beam position.

Rodríguez-Sánchez, V., Encina-Encina, L., Rodríguez-Ruiz, A., Sánchez-Carmona, R. (2015). Fisheries Research, **164**, 214–222.

Este estudio aporta ecuaciones de conversión horizontal para la especie barbo. Una especie importante en las asociaciones piscícolas europeas y de la que hasta ahora no se tenía información acústica. Estudiamos además el efecto que la selección de la longitud de pulso tiene sobre la energía devuelta por un pez, no encontrando diferencias en el sonido devuelto por los peces estudiados utilizando diferentes longitudes de pulso. Se ha trabajado también sobre dos de los problemas habitualmente encontrados durante el desarrollo de las ecuaciones de conversión: la estimación del ángulo de natación del pez al utilizar peces con natación libre y el tiempo necesario para la construcción de las ecuaciones. En el primer caso, se propone una nueva forma que integra toda la información sonora reflejada por un pez en movimiento y representa con fidelidad la orientación que éste adopta con respecto al plano del transductor. En el segundo caso, se ha estudiado el comportamiento del TS devuelto en diferentes posiciones del haz acústico. Los resultados han permitido la ampliación del área útil de adquisición de datos y la reducción del tiempo necesario para el desarrollo de las ecuaciones de conversión

ARTÍCULO II

Horizontal target strength of *Cyprinus carpio* using 200 kHz and 430 kHz split-beam systems.

Rodríguez-Sánchez, V., Encina-Encina, L., Rodríguez-Ruiz, A., Sánchez-Carmona, R.

En este estudio, las ecuaciones de conversión para la especie Cyprinus carpio (carpa común) se han desarrollado usando dos sistemas split-beam operando en distintas frecuencias (430 y 200 kHz). Se estudiaron por una parte, las posibles diferencias relativas a la frecuencia, tanto en la percepción de señales de peces como entre las ecuaciones de conversión. Por otra parte, se estudiaron las diferencias que las nuevas ecuaciones de conversión presentaban con otras desarrolladas para la misma especie pero con un sistema de haz doble. Los resultados mostraron que las diferencias son mayores cuando se comparan sistemas que funcionan con haces diferentes (haz doble vs. haz partido) que cuando se comparan frecuencias diferentes (200 y 430 kHz). Se estudió también la capacidad de detección y posicionamiento de las trayectorias de los peces ofrecido por las diferentes frecuencias. Los resultados obtenidos ponen de manifiesto que existen diferencias en la detección de peces según la frecuencia utilizada. Según nuestros resultados, para el estudio de peces individuales en sistemas superficiales y poco profundos y para la determinación de su posición en el espacio, la frecuencia de 200 kHz proporciona mejores resultados que la frecuencia de 430 kHz.

ARTÍCULO III

Do close range measurements affect the target strength (TS) of fish in horizontal beaming hydroacoustics?

Rodríguez-Sánchez, V., Encina-Encina, L., Rodríguez-Ruiz, A., Sánchez-Carmona, R. (2015). Article in press: Fisheries Research (2015), http://dx.doi.org/10.1016/j.fishres.2015.03.020

En este artículo se estudia y discute la efectividad de los muestreos de hidroacústica horizontal realizados a corta distancia. Se estudian los efectos del campo cercano y el comportamiento del TS con la distancia. Encontramos que el campo cercano teórico puede estar sobreestimado ya que se calcula a partir de la longitud del pez. Este campo cercano podría calcularse a partir de la longitud de la vejiga natatoria que es el órgano responsable de la mayor parte de la energía devuelta por un pez. Nuestros resultados muestran que recalculando el campo cercano en base a la longitud de la vejiga natatoria, se reduce la distancia de seguridad teórica que se aconseja evitar para la toma de datos acústicos y aumenta el volumen disponible para el análisis acústico sin que el TS sufra variaciones. Observamos, además, que para un mismo individuo el TS es estable en los primeros 12 metros de muestreo. Esto prueba que los datos hidroacústicos grabados con orientación horizontal son estables en las distancias usualmente utilizadas y refuerza el uso de esta técnica para el estudio de sistemas acuáticos someros.

DISCUSION GENERAL

En los ecosistemas acuáticos el estudio de los peces es fundamental para entender su funcionamiento y ayudar a la gestión y/o conservación del ecosistema. Entre las técnicas disponibles para el muestreo de las comunidades de peces destacamos por sus numerosas ventajas la técnica hidroacústica. Su uso está ampliamente extendido y aceptado por la comunidad científica por lo que actualmente se está trabajando para la normalización de la técnica (CEN, 2009). No obstante, la aplicación de la hidroacústica en su orientación horizontal para el estudio de los ecosistemas acuáticos superficiales y poco profundos es de reciente incorporación. Por ello, para desarrollar los protocolos de uso y aplicación de la técnica en orientación horizontal son necesarios trabajos que comparen sistemas hidroacústicos diferentes y estudien el comportamiento del sonido en el agua en diferentes situaciones.

En un estudio hidroacústico, la elección de una ecuación de conversión TS-longitud adecuada es fundamental para la obtención de estimas precisas del tamaño y biomasa de los peces (Boswell and Wilson, 2008; Boswell *et al.*, 2008). Así la comparación realizada en el artículo I entre la ecuación de conversión horizontal desarrollada para la especie barbo y las ecuaciones de conversión horizontal disponibles para otras especies (Kubecka y Duncan, 1998; Burwen y Fleischman, 1998; Frouzova y Kubecka, 2004; Frouzova *et al.*, 2005), pone en evidencia que el tamaño estimado para un barbo varía según la ecuación de conversión utilizada y se subestima en todos los casos. En el artículo III, se confirma que las diferencias entre ecuaciones se deben principalmente a las especies muestreadas con independencia de la metodología utilizada para la construcción de las ecuaciones.

En cuanto a las cuestiones relacionadas con la identificación de especies utilizando sistemas hidroacústicos, coincidimos con Frouzova *et al.* (2005) en que con las ecosondas actualmente disponibles y utilizando especies parecidas anatómicamente como las estudiadas en este trabajo, la hidroacústica horizontal no es útil para la identificación de las especies. No obstante, al existir diferencias significativas entre las ecuaciones de conversión por especies, se aconseja la utilización de ecuaciones específicas siempre que sea posible. Aplicar una ecuación de conversión adecuada nos servirá, por ejemplo, para determinar con mayor precisión las informaciones referentes a la distribución de tamaño de los peces, datos fundamentales para estudios de flujo energético, crecimiento o producción. Las ecuaciones desarrolladas en esta Tesis Doctoral posibilitarán la realización de estudios de comportamiento y migración para las especies barbo y carpa en ecosistemas acuáticos poco profundos y en aguas superficiales.

Por otra parte, la elaboración de las ecuaciones de conversión no es tarea fácil. En el trabajo realizado en el artículo I de esta Tesis Doctoral se ofrecen una serie de mejoras orientadas a la facilitación de la construcción de las mismas. La tendencia actual para la construcción de ecuaciones es realizarla con peces con natación libre ya que los datos obtenidos representan más fielmente la realidad que podemos encontrar en los ecosistemas naturales (McClathie *et al.*, 1996; Boswell y Wilson, 2008). Hasta el momento, no existe un método establecido y generalizado para calcular el ángulo de natación del pez (Huse y Ona, 1996; Pedersen *et al.*, 2009). Este trabajo presenta un método de cálculo que ha demostrado ser representativo de la orientación real del pez. El nuevo método incorpora toda la información que emite un pez y la integra en una recta de regresión que resume su movimiento. Coincidiendo con estudios previos (Pedersen *et al.*, 2009; Jech, 2011; Rodríguez-Sánchez *et al.*, 2015a) consideramos que el cálculo de la orientación del pez es fundamental para los estudios hidroacústicos realizados con aplicación horizontal, y en este sentido, establecer un método como el desarrollado en esta Tesis Doctoral para su uso generalizado, contribuirá a la estandarización de los estudios hidroacústicos.

Además, se ha trabajado para reducir el tiempo necesario para la construcción de ecuaciones de conversión. La superficie del haz principal útil para el análisis está teóricamente limitada a los primeros -3dB de caída del sonido desde el centro acústico (0 dB) (Simmonds y MacLennan, 2005). En el artículo I se han estudiado las posibles variaciones que pueden aparecer en el TS devuelto por un pez cuando se aumenta la superficie del haz teórico principal desde los -3dB a los -5dB de caída sonora. Coincidiendo con los resultados de

Brede *et al.* (1990) las comparaciones realizadas en nuestros estudio demuestran que no existe una variación significativa en el TS de las trayectorias encontradas en la parte central del haz principal (considerada desde 0º hasta los 3.5º) con el TS obtenido por una trayectoria localizada en la parte exterior del haz principal (considerada desde los 3.5º del centro del haz hasta los 4.5 º). Estos resultados confirman que un leve aumento en el área disponible para el análisis permite reducir el tiempo necesario para la adquisición de datos en los procesos de construcción de ecuaciones. Este aumento debe hacerse con cautela y no lo debemos utilizar para analizar datos hidroacústicos grabados en sistemas naturales, ya que esto podría provocar ciertas desviaciones en los resultados de densidad y biomasa. Por lo tanto, sólo se recomienda aumentar el área disponible del haz principal en los estudios realizados para la construcción de ecuaciones de conversión en condiciones similares a las presentadas en este estudio.

En el artículo I se demuestra además que para el sistema Simrad de haz partido a 200 kHz, no es necesario realizar ecuaciones de conversión específicas para las longitudes de pulso de 0.128 ms y 0.256 ms. Estos resultados coinciden con los obtenidos en la comparación de sistemas de haz doble realizada por Kubecka (1995) o con los obtenidos por Boswell y Wilson (2008) y Godlewska (2004), utilizando sistemas de haz partido con frecuencias diferentes a las aplicadas en este estudio. Los resultados demuestran que las mediciones de TS son estables independientemente de la longitud de pulso seleccionada para el muestreo. Igualmente se confirma que las estimaciones de densidad y biomasa de peces realizadas en ecosistemas acuáticos utilizando longitudes de pulso diferentes son directamente comparables.

Sin embargo, las ecuaciones de conversión presentan diferencias notables dependiendo del tipo de haz con que trabaje el sistema utilizado (haz doble o haz partido). En el artículo II, se comprueba que cuando convertimos los valores de TS obtenidas por un sistema determinado existen diferencias importantes si utilizamos las ecuaciones desarrolladas para un sistema de haz doble o las desarrolladas para un sistema de haz partido. Estos resultados coinciden con los obtenidos en experimentos previos de Traynor y Ehrenberg (1990) o Ehrenberg y

Torkelson (1996). Las diferencias en las estimas de longitud son notables y, por ende, serán también importantes las que encontraremos en las estimas de biomasa. Por tanto, para evitar desviaciones en las estimas de biomasa, las ecuaciones aplicadas en la conversión de los datos acústicos deben ser específicas para el tipo de haz utilizado.

Por otra parte la frecuencia utilizada también influye en el TS, aunque en menor medida que en tipo de haz aplicado. Las ecuaciones de conversión realizadas para las frecuencias de 200 y 430 kHz fueron significativamente diferentes. Coincidiendo con los resultados de Kubecka y Duncan's (1998), la frecuencia de 430 kHz registró siempre valores de TS menores que los obtenidos por la frecuencia de 200 kHz. Además, las trayectorias de peces seleccionadas para la comparación entre frecuencias presentaron un menor número de ecos en la frecuencia de 430 kHz y el TS de los peces insonificados fueron más bajos. Estas diferencias podrían explicarse por la directividad que presenta cada frecuencia, más alta en sistemas de mayor frecuencia (Horne y Clay, 1998). Su efecto puede producir pérdidas en la recepción de la energía devuelta por un pez y como consecuencia, una disminución en el TS recibido. Aunque Love (1971) recomienda la frecuencia de 430 kHz para utilizarse en situaciones de muestreos en distancias cortas, los resultados obtenidos en esta Tesis Doctoral demuestran que para las especies estudiadas (barbo y carpa), la frecuencia de 200 kHz ofreció mejores resultados en la recepción y posicionamiento de señales acústicas.

Pese a estas diferencias, la relación entre frecuencias se mantuvo constante a lo largo de las tallas y orientaciones estudiadas, por lo que las estimaciones de densidad o biomasa obtenidas por sistemas de diferente frecuencia, pueden ser directamente comparables aplicándoles un factor de corrección. Esto coincide con los resultados obtenidos en estudios previos realizados en sistemas naturales donde frecuencias similares, por ejemplo 70 kHz y 120 kHz, presentan estimas de densidad equivalentes (Godlewska *et al.*, 2009). No obstante, frecuencias mayores presentan diferencias más notables, especialmente en casos de alta densidad piscícola (Wanzenbock *et al.*, 2003; Guillard *et al.*, 2004). Los resultados obtenidos en el artículo II confirman los publicados anteriormente, concluyéndose que diferentes frecuencias reciben la energía de los peces de diferente forma aunque no siempre estas diferencias quedan reflejadas en las estimas de densidad y biomasa proporcionadas por los sistemas. Además, en este artículo se demuestra que sistemas con alta frecuencia pueden ser menos útiles para estudios de hidroacústica horizontal, donde la información referente al ángulo de natación del pez es fundamental para las estimaciones tamaño y biomasa. Se sugiere por tanto, aplicar frecuencias de 200 kHz o menores cuando se llevan a cabo mediciones de hidroacústica horizontal a corta distancia.

Todas las comparaciones presentadas son importantes para la determinación de los aparatos a utilizar en los muestreos hidroacústicos y para establecer los protocolos de uso de la técnica, necesarios a la luz de las nuevas normativas europeas (CEN 2009). Además, en los ejercicios de intercalibración exigidos por la DMA, estos resultados nos sirven de ayuda para la correcta interpretación y comparación de los datos acústicos proporcionados por sistemas diferentes.

Finalmente, este estudio trabaja sobre una cuestión importante en la hidroacústica horizontal que influye directamente en su aplicabilidad para el estudio de los sistemas someros: la distancia. Según los resultados presentados en el artículo III, los TS horizontales de grandes peces no dependen de distancias estudiadas (6, 9 y 12 m). Coincidiendo con los resultados de Dawson *et al.* (2000) y Knudsen *et al.* (2004) concluimos que las fórmulas estándar para el cálculo del campo cercano sobrestiman la proporción de espacio que ocupa. Por ello se propone que los cálculos del campo cercano se realicen en base a la longitud de la vejiga natatoria ya que ésta es responsable de la mayor parte de la energía retrodispersada por un pez (Foote, 1980; Hazen y Horne, 2003). Por otra parte, el campo cercano calculado a partir de la vejiga natatoria es menor que el calculado a partir de la longitud total del pez, lo que aumenta indirectamente el volumen de agua disponible para el análisis. Esto confirma, una vez más, que la hidroacústica horizontal es una herramienta útil para el estudio de los peces en sistemas someros y superficiales realizados a corta distancia.

Otro de los problemas discutidos en este artículo III tiene que ver con la insonificación de peces grandes (de longitud mayor al haz acústico) a corta distancia. Se podría esperar que el TS de un pez grande (de longitud mayor que la anchura del haz principal) insonificado a corta distancia fuera diferente al obtenido por ese mismo pez a una mayor distancia, donde la anchura del haz permitiera su completa insonificación. Nuestros resultados mostraron que el TS medio de un pez grande insonificado a corta distancia permanecía estable a lo largo de las distancias estudiadas, por lo que parece que si a una distancia determinada la vejiga natatoria queda incluida en el haz principal, el sonido de este pez no varía aunque su cuerpo no quede completamente insonificado.

Los resultados obtenidos en esta Tesis Doctoral destacan la utilidad y la precisión de las estimaciones de densidad y biomasa obtenidas con el uso de la hidroacústica horizontal. Clarifican las especulaciones anteriores y representan un paso adelante en la comprensión del comportamiento de sonido para la detección de peces en los sistemas poco profundos. Consideramos que la hidroacústica horizontal es una técnica que presenta un futuro prometedor en los estudios de peces. En este sentido, la técnica podría ser muy útil en los trabajos rutinarios de manejo de sistemas poco profundos como son las balsas de cultivo de peces utilizadas en acuicultura y sistemas afines.

PERSPECTIVAS DE APLICACIÓN DE LA HIDROACUSTICA HORIZONTAL

La hidroacústica horizontal presenta perspectivas muy interesantes en el ámbito de la ecología de peces. Existen numerosos sistemas acuáticos poco profundos que son difíciles de muestrear para conseguir valores cuantitativos absolutos. La hidroacústica horizontal podría ser el complemento perfecto a las artes de pesca tradicionales. Con el uso de esta técnica, sería posible eliminar algunos de los sesgos referidos a la selectividad de las técnicas tradicionales en los estudios censales de la ictiofauna en ecosistemas someros. También para el estudio de masas de agua profundas, como los embalses y lagos, la hidroacústica horizontal se presenta como un complemento a las estimaciones proporcionadas con hidroacústica vertical ya que aportaría información de las zonas litorales, donde la hidroacústica vertical no es eficiente. El uso combinado de ambas aplicaciones (vertical-horizontal) sería lo más recomendable ya que permitiría estudiar el sistema completo, incluyendo en las estimaciones tanto los peces que habitan en las zonas profundas como los que habitan en las zonas someras y los estratos superficiales.

La hidroacústica horizontal se perfila también como una técnica muy útil en los estudios de migración de peces, sustituyendo las técnicas de captura y recaptura tradicionales. De hecho desde hace unos años se utilizan las técnicas hidroacústicas para estudios de migración de salmónidos en ríos de Norte América y Europa (Steig and Iverson, 1998; Ransom *et al.*, 1996). No obstante, el sondeo hidroacústico en zonas muy someras, donde se presentan obstáculos a la detección de peces (vegetación sumergida, reverberación de superficie y fondo,…), requiere aun de más ensayos y calibraciones específicas para la correcta interpretación de los resultados. En este sentido se deberá seguir trabajando para la mejora de la técnica y para determinar las limitaciones que pueda presentar.

Igualmente, la hidroacústica horizontal podría utilizarse para la gestión de los sistemas someros utilizados en acuicultura para la cría de peces. La acuicultura necesita de censos periódicos para la determinación del tamaño y número de peces y utilizar métodos acústicos podría ser una forma de evitar la manipulación directa y el estrés que para los animales conlleva un muestreo extractivo. De hecho, la base metodológica desarrollada en esta Tesis Doctoral se está utilizando actualmente para el desarrollo de metodologías aplicables a la gestión y manejo de las balsas de acuicultura. El objetivo de dicho estudio es desarrollar un protocolo de muestreo para determinar el número y tamaño de los peces en balsas de cultivo de peces en tierra. Todo ello para incorporar la técnica hidroacústica en las rutinas de manejo y gestión de las piscifactorías de este tipo.

Podemos resumir que la aplicación de la hidroacústica horizontal para el estudio de sistemas someros está recién implantada y requiere de estudios que contribuyan a su desarrollo y que proporcionen herramientas para su correcta comprensión e interpretación. En este sentido, esta Tesis Doctoral contribuye sustancialmente al desarrollo y aplicación de estas técnicas. Hemos estudiado el efecto de algunos de los parámetros más importantes en la conversión de los resultados acústicos como son la especie estudiada, la frecuencia aplicada para el estudio, el sistema utilizado para la obtención de datos y la longitud de pulso aplicada. En todos ellos encontramos que la técnica ofrece excelentes condiciones para ser utilizada con éxito en estudios de peces realizados en sistemas superficiales y poco profundos.

En este trabajo se subraya la importancia que tiene utilizar una ecuación de conversión TS-longitud adecuada para la especie y el sistema (haz doble-haz partido) utilizado. Asimismo encontramos que las frecuencias altas (430 kHz) pueden resultar menos adecuadas para el estudio de estos ecosistemas someros. La frecuencia de 200 kHz posiciona mejor los ecos de los peces y, por tanto, el ángulo de natación del pez es más real, lo que evita desviaciones en las estimas de tamaño y biomasa. Por otra parte, encontramos que la hidroacústica horizontal presenta un buen funcionamiento en los primeros metros de muestreo, permitiendo medidas estables de sonido. Este resultado es muy alentador ya que cuando aplicamos estas técnicas la información se extrae de los primero metros de muestreo.

Decir que los trabajos de este tipo son necesarios para seguir avanzando en la mejora y aplicación de la técnica. Por una parte, nos ayudan a entender el comportamiento del sonido aplicado a la detección de peces y esto nos permite tener herramientas científicas para justificar la elección de los sistemas a utilizar. Por otra parte, nos proporcionan información útil para los ejercicios de intercalibración requeridos por la Directiva Marco de Agua.

La hidroacústica horizontal presenta nuevas perspectivas de uso en el estudio de las poblaciones de peces en los ecosistemas acuáticos. Puede ser altamente útil para estudios de migración de especies en sistemas ribereños. Además, su uso combinado con hidroacústica vertical nos permitirá obtener estimas de densidad más completas y cercanas a la realidad del ecosistema acuático. Por otra parte la hidroacústica horizontal podría ser útil para la gestión y manejo de las poblaciones de peces balsas de cultivo y otros sistemas de producción, en los que conocer la abundancia de peces a tiempo real es de vital importancia.

REFERENCIAS en página ………………………………………………………..58

CONCLUSIONES

- La señal acústica horizontal (TS) de barbos y carpas varía según la orientación del pez con respecto del haz acústico por lo que la información referente al ángulo de natación debe quedar incluido en las ecuaciones de conversión TS-longitud.

- Para mejorar la precisión de las estimas de biomasa son necesarias el uso de ecuaciones específicas, ya que las ecuaciones de conversión TS-longitud para barbos y carpas son significativamente diferentes.

- Se aconseja la aplicación generalizada del método desarrollado para el cálculo de natación del pez, pues representa la orientación real del pez e integra toda la información acústica reflejada en su movimiento.

- Para la construcción de ecuaciones de conversión en condiciones similares a las presentadas en este estudio, podemos aumentar el área del haz principal hasta los -5dB del centro acústico sin variar el TS del pez insonificado.

- Para el sistema Simrad de haz partido a 200 kHz, no es necesario realizar ecuaciones de conversión específicas para las longitudes de pulso de 0.128 ms y 0.256 ms.

- Los resultados de biomasa calculados a partir de datos hidroacústicos mejoran cuando las ecuaciones aplicadas en la conversión son específicas para el tipo de haz utilizado (haz doble o haz partido) y para la frecuencia aplicada.

- Para el estudio hidroacústico en sistemas someros de las especies de barbo y carpa se recomienda la frecuencia de 200 kHz, ya que ofrece mejores resultados en la recepción y posicionamiento de señales acústicas.

- La fórmula estándar para el cálculo del campo cercano sobrestima la proporción de espacio que ocupa. Se propone que los cálculos del campo cercano se realicen en base a la longitud de la vejiga natatoria por ser ésta responsable de la mayor parte de la energía retrodispersada por un pez.

- En hidroacústica horizontal, el TS medio de un pez grande insonificado a corta distancia permanece estable en los primeros metros de insonificación.